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The Behavioral and Neuronal Mechanisms of the Shadow Response and its Role in the Diel Vertical Migration of a Hydromedusan (*Polyorchis penicillatus*)

by

© Stuart A. Arkett

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The Behavioral and Neuronal Mechanisms of the Shadow Response and its Role in the Diel Vertical Migration of a Hydromedusan (*Polyorchis penicillatus*) submitted by Stuart A. Arkett in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

A.N. Spencer
Supervisor

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External Examiner

Date May 30th 1985

Abstract

The hydromedusan ***Polyorchis penicillatus*** was observed to make a small amplitude diel vertical migration. During the day, over 85% of the individuals were observed within 1 m of the bottom, while at night, medusae migrated several meters up into the water column. This movement occurred concomitantly with the emergence of many of the major taxa of demersal zooplankton whose diel activity was quantified by emergence traps. Food boli contents collected from ***Polyorchis*** showed some selectivity for large, fast-moving demersal plankters and low capture and utilization of invertebrate larvae. Diel shifts in feeding behavior and water column position enable ***Polyorchis*** to feed efficiently on high densities of demersal plankters at all times. Medusae may be exempt from migration constraints imposed by visual predators and are thus able to move into areas of high prey density to supply energetic requirements. Hydromedusae, such as ***Polyorchis***, are shown to use a variety of factors, such as tentacle position and swimming speed, in an "ambush" strategy to optimize prey encounters.

Treadmill experiments show that the swimming frequency of ***Polyorchis*** is directly proportional to the rate of decrease in light intensity. Slow, continuous increases in light intensity cause an inhibition of swimming and progressive "crumpling". These slow changes in light intensity, which are similar to the changes during sunset and sunrise, may initiate the diel movements of ***Polyorchis*** in the field. The threshold rate of light intensity decrease for the shadow response appears to ensure that the upward movements would begin after sunset. Similarly, the threshold rate of light intensity increase necessary to elicit the inhibition of swimming probably ensures that sinking occurs by sunrise. ***Polyorchis*** responds to rapid white light OFF-ON shadows with one swimming contraction, with a peak monochromatic response at around 450-550 nm. These results suggest that the shadow response of ***Polyorchis*** is not used in predator avoidance since the response would not provide effective escape from a potential predator. Most of the photic responses of ***Polyorchis*** show size- (age) related differences. These differences may result in ontogenetic changes in feeding behavior and distribution.

Electrophysiological experiments show that the shadow response of *Polyorchis* is a demonstrable reflex and is characterized by: 1) morphologically and physiologically identifiable reflex arc components, ("O" system, "B" system, SMNs, tentacle myoepithelium, and swimming myoepithelium); 2) a predictable sequence of events following a shadow ("O" system hyperpolarization, followed by "B" system and SMN spiking which produce tentacle and swimming myoepithelium contractions, respectively; and 3) graded responses of the components with respect to the rate of change in light intensity. The graded response of the "O" system to photic stimuli appears to be similar to ERG studies of *Polyorchis*. This together with the finding that "O" system recordings can be made from within the ocelli suggests that the receptor cells of the ocelli are the terminal outgrowths of the "O" system. This is the only system found to be photosensitive. The electrically-coupled "O" system is of primary importance in the shadow response, functioning as the photoreceptor, transmitting photic information to efferent groups ("B" system, SMNs) and may integrate light information in a way analogous to the receptor cells of the vertebrate retina. A 28-30% instantaneous reduction in light intensity appears to be the threshold for the shadow response because percent reductions less than this failed to yield a response. The greatest response occurs after 100% reductions in light intensity and usually consists of only a few rapid swimming contractions. This again demonstrates the ineffectiveness of the shadow response in predator avoidance.

Sinking rate experiments show that individual medusae sink at rates between 18 cm/min and 60 cm/min with smaller individuals sinking slightly slower than larger individuals. Daytime adapted medusae sink at a significantly faster rate than nighttime individuals. These results suggest that diel changes in the buoyancy of *Polyorchis* may contribute to diel vertical migration after its initiation by the shadow response and also maintain daytime and nighttime water column position.

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I. INTRODUCTION

Rationale for the Study

There are many definitions of behavior, but most suggest that behavior is any observable movement or process exhibited by an organism in response to some self-perceived change either within itself or in its surroundings. This broad definition encompasses a wide variety of processes at many levels of complexity, but one of the simplest forms of behavior is the reflex (Kandel 1976). Reflexes are essentially inheritable, unconscious, neuromuscular adjustments (Romanes 1883). This simple form of behavior is highly stereotypic and the various components involved in a reflex can be identified. These properties of reflexes have interested neuroethologists for a long time. Indeed, these properties appear to be essential to neuroethologists because "the only way a behavioral act can be fully understood is in terms of the actual functioning of the individual neurons producing the output / commands and of the circuits in which they operate." (Hoyle 1984). This conclusive statement by Hoyle may be an omen for the cellular studies of more complex behavior involving volitional action (Rose 1980). Thus, it would seem most profitable to our understanding of behavior to examine the various components of reflexes in less complex organisms. Indeed, the possibility of elucidating the fundamental properties of simple behavior is often the rationale for using invertebrates as model systems. By this reasoning then, it appears that our best chance for fully understanding behavior would be to examine the most simply organized animals that possess an unquestionably defined nervous system; the cnidarians.

There has been a long-standing interest in cnidarian (and particularly medusan) behavior and nervous systems. The classic works of Hertwig and Hertwig (1878) and Romanes (1876, 1877) founded many current ideas about hydromedusan behavior and nervous organization. Notably, many of their experiments were designed to determine the importance of the marginal nerve-rings and their central role in the coordination of behavior. In addition to describing morphological differences between the two nerve-rings, both Romanes and the Hertwigs made functional distinctions between the rings. They reasoned that the lower (inner) nerve-ring, which is composed of relatively large fibers, was primarily motor in nature because of its location close to the swimming musculature on the subumbrellar surface of the bell. The upper (outer) nerve-ring is composed of a larger number of relatively small nerve fibers and the Hertwigs and Romanes reasoned that because of its close proximity to sensory structures (e.g., ocelli, tentacles), the outer nerve-ring was probably important in the integration of sensory information. The confirmation of these early postulates about the function of these two centralized nerve-rings and their roles in the coordination of movements has been a focus of attention of coelenterate neurobiologists since then.

Medusan reflexes and the importance of the nerve-rings to these simple behaviors have been known for about 100 years. Romanes (1885) often referred to various behaviors as "reflex actions" and demonstrated that the execution of these behaviors depended upon the marginal nerve-rings. Even Charles Sherrington (1906) extensively referred to medusae while describing properties of simple reflexes and likened the causes of medusan swimming to his concept of "reflex irradiation". This idea that complex movements are generated by some orderly combination of reflexes has long since been discarded, giving way to the current dogma of central control (Delcomyn 1980; Kristan 1980). However, the reflex may still be a useful concept even though it holds a dated stigma. Modern definitions of a reflex usually include: 1) identification of reflex arc components, which minimally include a receptor, a centralized integrator, efferent neurons, and effector organs; 2) a predictable sequence of events; and 3) a graded response to a specific stimulus. The last two characteristics of a reflex have been known in hydromedusae since the Hertwig's time. Even the morphology and organization of medusan receptors, muscles, and neurons in the nerve-rings have been documented for some time (Hertwig and Hertwig 1878; Linko 1900; Little 1914). However, the physiological identification of reflex arc components, by intracellular recordings, has been lacking, until only very recently.

The first intracellular recordings from a hydromedusan were made from the large neurons within the inner nerve-ring of *Polyorchis penicillatus*. These neurons directly control swimming muscle contractions (Anderson and Mackie 1977) and have been called swimming motor neurons (SMNs) (Spencer 1978). Subsequent work by Anderson (1979), Spencer (1981, 1982) and Spencer and Satterlie (1980, 1981) continued to examine the properties of the SMNs as well as the swimming muscles. These studies confirmed the ideas of the Hertwigs and Romanes that the inner nerve-ring was primarily involved in motor control. However, the outer nerve-ring remained a black box, with known afferents entering it and post-synaptic activity observed in the SMNs coming from it. In 1984, Spencer and Arkett identified two discrete neuronal networks in the outer nerve-ring of *Polyorchis* by intracellular recordings. This was a significant advance because for the first time, intracellular recordings were possible from the centralized outer nerve-ring, which contain neuronal networks pre-synaptic to the SMNs and post-synaptic to sensory organs. This advance enabled us to begin to understand how sensory information might be integrated by a simple, radially symmetrical nervous system and used to alter locomotory patterns and other behavior.

That sensory information is important to the alteration of one behavior, notably swimming activity, is evidenced by the additional finding of Anderson and Mackie (1977) that *Polyorchis* responds to a rapid reduction in light intensity in the form of a rapid burst of SMN action potentials and thus swimming contractions. Anderson and Mackie (1977) attributed this predictable response to shadows to the ocelli and they suggested that this marked response may be used in predator avoidance. Additionally, they found that the SMNs were photosensitive, that is, the spiking frequency and membrane potential of the SMNs were directly related to the ambient light intensity. From this finding, Anderson and Mackie (1977) suggested that swimming and thus diel vertical migrations of medusae may be controlled by the ambient light intensity. These findings generated many of my initial questions in this study.

The main purpose of this thesis is to characterize the photic behavior of *Polyorchis penicillatus* and to relate this behavior to its diel patterns of activity in the field. To this end , the thesis is composed of five papers, each one dealing with an aspect of the photic behavior of *Polyorchis*. In the first (Chapter 2; Arkett 1984), I demonstrate that *Polyorchis* is a demersal hydromedusan, spending much of its time on or near the bottom during the daytime and migrating a short distance into the water column at night. This typical diel vertical migration pattern coincides with the emergence of many demersal zooplankters. These plankters are the principal prey items found in food boli from

Polyorchis. I propose in this chapter that the feeding behavior and diel movement patterns of *Polyorchis* may optimize encounters with the demersal zooplankton. In Chapter 3, I examine various photic responses of wholly intact *Polyorchis* on a "jellyfish treadmill". Here I demonstrate that very rapid reductions in light intensity do not alter the normal swimming frequency of *Polyorchis*. Slow, continuous decreases and increases in light intensity do alter swimming frequency, causing near continuous swimming and "crumpling", respectively. I propose that these changes in swimming frequency may thereby initiate diel vertical migrations. I also propose that the response of *Polyorchis* to rapid light intensity reductions, the shadow response, does not function in predator avoidance. In Chapters 4 and 5, I attempt to demonstrate that the shadow response of *Polyorchis* is a simple reflex. I do this by showing the cellular behavior of various components involved in the shadow response, using photic stimuli similar to those used in the whole animal experiments in Chapter 3. I demonstrate that the reflex components are identifiable, that there is a predictable sequence of events in the response, and that the response is graded with respect to a specific stimulus. I also propose a mechanism by which the centralized "O" system may integrate photic information. In Chapter 6, I demonstrate that *Polyorchis* shows some diel changes in its sinking rate. Slower sinking rates at night suggest that buoyancy changes may augment upward movements due to the shadow response and help maintain a shallower water column position throughout the night. Chapter 7 consists of my concluding remarks.

This thesis is an attempt to explain a simple behavior in a relatively simple organism by using a variety of techniques and approaches. I have examined the photic behavior of *Polyorchis* through observations in the field, in whole-animal controlled laboratory experiments, and through the cellular properties of the neuronal systems that control this behavior. The greatest strength of this thesis lies in this integrative approach; this may be the only way to understand fully any behavior.

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II. FIELD DISTRIBUTION AND BEHAVIOR

Diel Vertical Migration and Feeding Behavior of a Demersal Hydromedusan(*Polyorchis penicillatus*)

Introduction

Gelatinous zooplankters have often been overlooked in descriptions of diel vertical migrations even though they are frequently abundant members of the plankton. This may be due in part to their frailty and destruction when collected in plankton nets. There are, however, a few detailed accounts of vertical migrations of medusae. Russell (1925), Moreira (1973), and Mills (1982), all using plankton tows, reported distinct diel vertical migrations of numerous species of hydromedusae. Yasuda (1973) found that a scyphomedusa, *Aurelia aurita*, rises during the day and sinks at night, a reversed diel migration. These studies used plankton nets often taking samples tens of meters apart, which may underestimate the numbers and distort the population distribution. Medusae that migrate only short distances or are close to the bottom may be considered nonmigratory or are missed altogether with this sampling method. Hamner et al. (1975) and Mackie and Mills (1983) have circumvented these problems and have shown the usefulness of direct field observations for studying migrations, aggregations, and behavior of medusae and other gelatinous zooplankton. Studies using large containers (Mackie et al. 1981; Mills 1983) have also been useful in observing diel migration of medusae. Although it is clear that some medusae do migrate throughout the water column in a coordinated manner, the adaptive value of these migrations has not been demonstrated.

One possible advantage of diel migration and concurrent changes in feeding behavior may be to increase prey encounters. Medusae exhibit a wide variety of feeding behaviors (Fraser 1969; Mackie 1980; Mills 1981; Bailey and Batty 1983), but most medusae feed using some form of "sink-fishing", a behavior in which the tentacles are extended and catch prey on contact as the individual sinks through the water column. The number of prey encountered and the types of prey captured by medusae then depend on tentacle number, tentacle orientation, behavior during sink-fishing bouts, rate of sinking, and water column position. Because most medusae are purely planktonic, they feed using some form of sink-fishing, almost exclusively on small plankters. Yet, some medusae have specialized to use other resources. One such medusa is *Haliclystus*, which has adopted a semisessile mode and relies on drifting plankton. The small trachymedusan *Tesserogastria* rarely swims; instead it occupies mud surfaces and eats small infauna (Hesthagen 1971). The scyphomedusan *Cassiopeia* lies on the bottom with its oral side facing upward. In this position, *Cassiopeia* swims, causing water currents, which presumably carry sediments and food, to flow over the oral arms (Hyman 1940). Hydromedusae of the genus *Polyorchis* also spend much of their time at or near the bottom, but unlike other bottom-dwelling medusae, they are also active swimmers. The food boli contents of *Polyorchis karafutoensis*, found in the western Pacific, are indicative of this bottom-dwelling behavior. Large, demersal plankters as well as purely planktonic forms are regularly found within its manubrium (Zelickman 1976). The hydromedusan *Polyorchis penicillatus*, commonly found near the bottom of shallow, muddy bays on the west coast of North America, also often has large demersal crustaceans in its manubrium. Mills (1983), using large cylinders, observed diel movements of *Polyorchis* and suggested that capture of and feeding on demersal plankters by *Polyorchis* may be facilitated by its movements in the water column. The diel migration of *Polyorchis* and its unusual bottom-affinity behavior may be a unique adaptation to utilize a resource not commonly exploited by other medusae. The purpose of this study was to determine the extent of the diel vertical migration of *Polyorchis* in the field and to correlate its movements with those of its principal prey.

I directly observed a small amplitude diel vertical migration of the hydromedusan *Polyorchis penicillatus*. The migration occurs concomitantly with that of demersal plankters, which are the principal prey of *Polyorchis* as determined from the contents of food boli. I relate some of its feeding behavior observed in the field to the feeding strategy used by ambush predators.

Methods and Materials

The study area in Bamfield Inlet, Bamfield,B.C. (48° 49' N, 125° 08' W) has a soft mud bottom with *Zostera marina* along the edges. Occasional macroalgal "islands" of *Agarum cribrosum, A. fimbriatum, Macrocystis integrefolia, and Laminaria saccharina* are found on an otherwise nearly homogeneous bottom. Depth of the sampling area ranged from 6-10 m, depending on the tide.

The vertical distribution of *Polyorchis* was determined visually using SCUBA. Vertical columns of water were defined by a weighted float-line marked at 1 m intervals and the bottom 1 m into 0.5 m intervals. Ten to twenty replicate columns were made at the study site at nine day (08:30-20:30) and nine night (20:30-08:30) periods during late July and August 1982. The position of each replicate column in the study area was randomly selected by a surface diver. For each replicate column a second diver moved vertically down and up the float line counting the number of individuals observed within 1 cubic meter. This area was defined by the float line and a PVC frame. A dive-light was mounted on the frame during the night and during periods of low visibility.

Behavioral observations of *Polyorchis* were made by divers during the day. Divers maintained neutral buoyancy and observed undisturbed single individuals for 10 minutes. The total number of swimming contractions during this time was recorded. A total of 39 individuals over 10 dives were observed at various times of the day.

Emergence traps, modeled after those designed by Alldredge and King (1980), were used to determine the composition and abundance of demersal zooplankton. Emergence traps, without the catch-bottles, were slowly lowered to the bottom site by divers. Replicate traps were placed less than 2 m from each other. Once a trap was on the bottom, the base was forced into the mud to ensure a seal from surrounding water. Catch-bottles filled with filtered (110 μm Nitex mesh) sea water were sealed and transported to the traps. Seals were broken and catch-bottles put into place. The catch-bottle opening was 0.5 m from the substratum. This procedure was followed throughout sampling to prevent disruption of the flocculent, bottom mud. At the end of a sampling period, the catch-bottles were removed from traps, immediately sealed, and transported to the surface. Bottle contents were filtered through 110 µm Nitex mesh and fixed in 10% formalin with rose bengal. The traps were repositioned, catch-bottles refilled, and replaced onto traps. Samples were sorted to major taxonomic groups, counted, and the total number of individuals per square meter was determined. A single sampling run consisted of two consecutive days yielding two night (20:00-08:00) and two day (08:00-20:00) samples with three trap replicates for each sample. Three such runs were made during late July and August 1982.

Ten to twenty individual ***Polyorchis*** having a visible food bolus were collected concurrently with each day and night sample. The food boli were removed from individuals and fixed in 10% formalin with rose bengal. Boli contents were later examined and nearly whole, identifiable individuals were counted.

Results

Migration Patterns of Polyorchis

Densities of 2 to 5 individuals per m^3 were regularly observed; however, large aggregations of up to 64 per m^3 were not uncommon. These large aggregations were most often found close to the bottom and clustered around *Agarum*. Most individuals counted were larger than 1.0 cm bell height. Consistent visual observations of smaller individuals proved too difficult.

The diel movements of *Polyorchis* were of low amplitude and generally confined to the area within several meters of the bottom (Fig. 1). There was an upward movement several hours after sunset which resulted in a more even distribution in the water column throughout the night. Just after sunrise, individuals were found very close to the bottom and usually remained in this position throughout the day. Pooled night and daytime samples (Fig. 2) show increases in the percentage of individuals higher in the water column at night, while during the day, over 85% of the individuals were within 1 m of the bottom.

Demersal Plankton Emergence

The major taxa of demersal plankters consistently showed two distinct emergence activity patterns (Table I). Those that emerged at night constituted the larger group with copepods caught in the greatest numbers. Most of the large, fast-moving demersal taxa (e.g., gammarid amphipods, cumaceans, ostrocods, two species of polychaetes) showed significant nighttime increases in abundance (Table I). Larval or juvenile stages of invertebrates were consistently captured in greater numbers during the daytime. Spionid larvae and veligers were often very abundant. Other organisms occasionally captured included cladocerans, larvaceans, and megalops larvae.

Food Bolus Contents

Figure 1. Mean percentage of individual *Polyorchis* sighted at each depth during selected sampling periods from 21 July to 27 August 1982 in Bamfield Inlet. The 6 sampling periods shown here were selected from the nine day and nine night periods to illustrate the general diel distribution of *Polyorchis*. Time of each sampling period is shown above the columns and the number of replicate columns for each sampling period is shown in parentheses. Sunset was approximately 19:00 and sunrise 06:00.

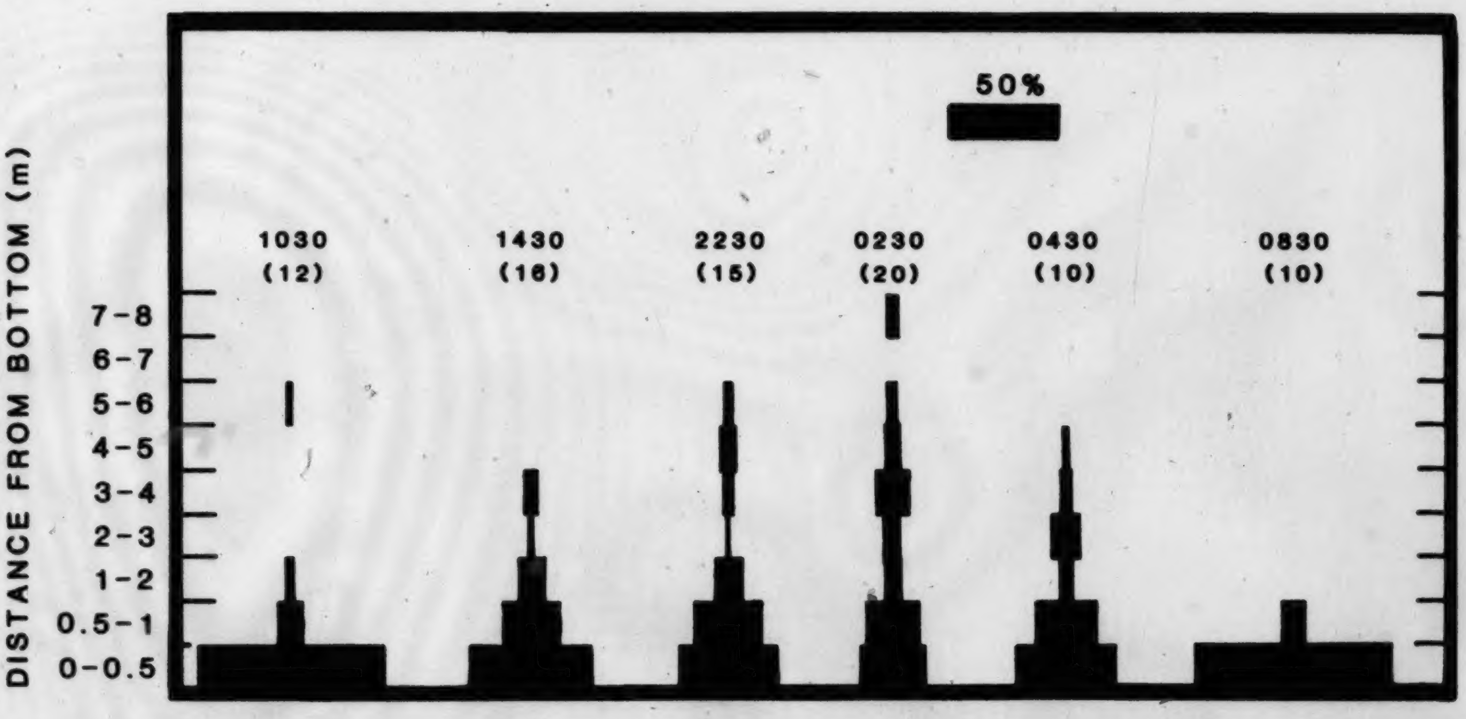
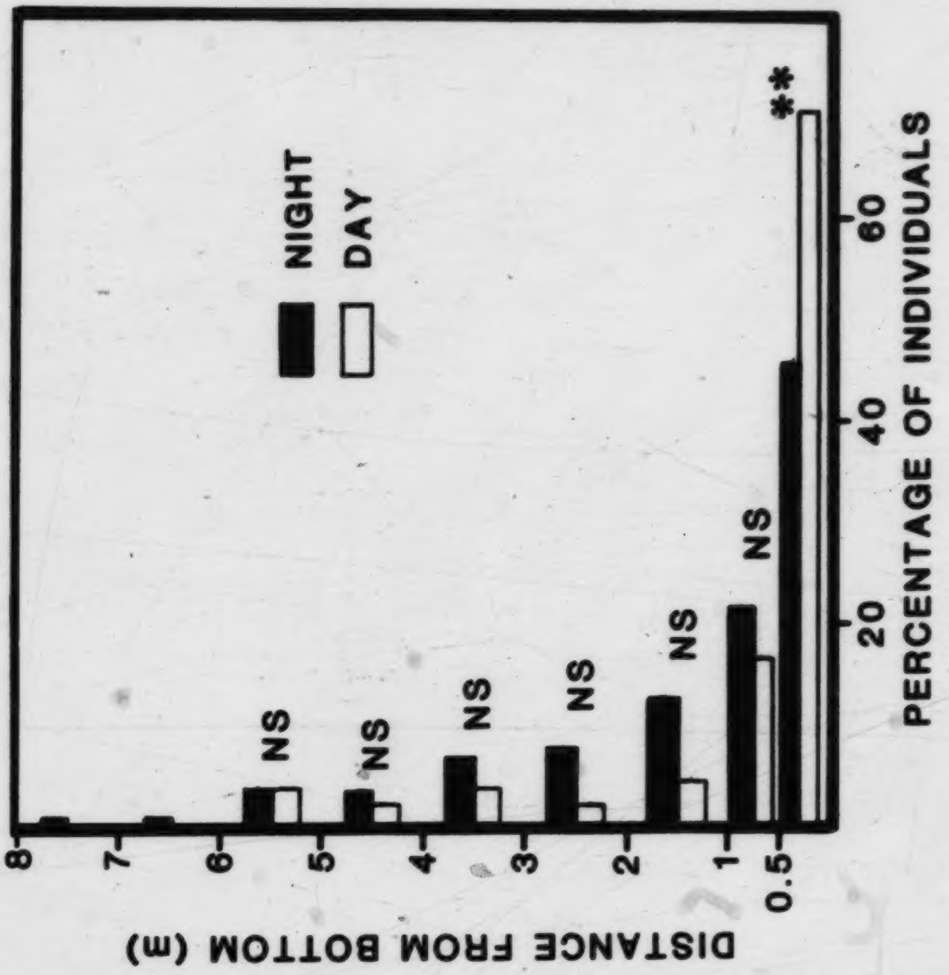

50%
1030
(12)
1430
(16)
2230
(15)
0230
(20)
0430
(10)
0830
(10)
DISTANCE FROM BOTTOM (m)
7-8
6-7
5-6
4-5
3-4
2-3
1-2
0.5-1
0-0.5

Figure 2. Mean percentage of individual *Polyorchis* sighted in the water column during nine day (08:00-20:00) and nine night (20:00-08:00) samples from 21 July to 27 August 1982 in Bamfield Inlet. Day-night comparisons between mean percentage of individuals at each depth were made using the Wilcoxon two-sample test (Sokal and Rohlf 1969) ** = $0.01>p>0.001$; NS = $p > 0.05$. Pooled number of column replicates for day and night were 100 and 111, respectively.


DISTANCE FROM BOTTOM (m)
8
7
6
5
4
3
2
1
0.5
PERCENTAGE OF INDIVIDUALS
20
40
60
■ NIGHT
□ DAY
NS
NS
NS
NS
NS
NS
**

Bolus contents consisted primarily of crustacean exoskeleton fragments tightly bound with mucus. Contents were in various stages of digestion with the greatest degree of digestion found in the proximal portion of the manubrium (nearest gonad attachment). Most of the intact contents were found in the distal portion of the manubrium; however, some items such as spionid larvae, veligers, ostracods, and cyprids were found intact at the proximal end as well.

Organisms within food boli were similar to those found in demersal trap samples, regardless of the time collected (Table I). Nighttime increases in numbers of cumaceans, ostracods, zoea larvae, and cyprids collected in traps, however, corresponded with increases in numbers of individuals present within food boli. Copepod fragments were present in all food boli, but no attempt was made to quantify day-night differences. Daytime food boli were almost totally devoid of groups with increased daytime activity, whereas, the presence of larvae within food boli increased at night. Caprellid amphipods and tanaids were consistently found within boli both during the day and night, but, these taxa were never collected in the traps. Other organisms occasionally found in boli included fish larvae, nematodes, and megalops larvae.

Feeding Behavior

During each 10 min. observation period, individuals of ***Polyorchis*** spent 90-95% of their time in a "sink-fishing" posture (Fig. 3, #1&9). Tentacles were held outward at their bases and more distal portions of tentacles dropped downward. Drifting through the water, individuals maintained this posture and swam at a very low frequency of 3-15 swimming contractions per minute. Smaller individuals swam at slightly higher frequencies than larger individuals (Fig. 4). I will refer to this low frequency swimming as "maintenance swimming" as the frequency was far too low to result in any net upward movement and merely maintained their position in the water column. During this maintenance swimming medusae usually remained at the same depth or sank very slowly.

The sink-fishing posture was disrupted by occasional contact with bottom obstructions (e.g., "islands" of ***Agarum***, logs, sea anemones) and usually resulted in 1-2 very rapid swimming contractions. These swimming contractions resulted in upward movements of several centimeters. Tentacles were often partially or fully contracted

TABLE I. Mean number (± 1 SE) of individuals $/m^2$ of bottom collected in demersal plankton emergence traps during day (08:00-20:00) and night (20:00-08:00) in Bamfield Inlet. Numbers have been pooled over three separate two-day sampling periods from late July and August 1982 with a total of 18 replicates for day and night samples. $** = p < 0.01$ $NS = p > 0.05$ The total number of identifiable prey items within food boli from *Polyorchis* collected at 08:00 for night and 20:00 for day samples is shown in parentheses. (p) indicates a presence, but total numbers not determined. Polychaete sp. 1 is *Ophiodromus pugettensis*, sp. 2 is from Opheliidae, sp. 3 is from Sphaerodoridae.

Taxon	Night		Day		
Calanoid copepods	3540 ± 645	(p)	2567 ± 573	(p)	NS
Cyclopoid copepods	282 ± 54	(p)	120 ± 16	(p)	**
Harpacticoid copepods	521 ± 71	(p)	278 ± 19	(p)	**
Gammarid amphipods	106 ± 18	(5)	6 ± 1	(10)	**
Cumaceans	9 ± 2	(33)	0.4 ± 0.5	(15)	**
Ostracods	22 ± 3	(25)	1 ± 0.5	(4)	**
Zoea larvae	132 ± 43	(36)	54 ± 9	(20)	NS
Cyprids	15 ± 3	(199)	9 ± 2	(94)	NS
Polychaete sp. 1	7 ± 2	(3)	1 ± 0.5	(4)	**
Polychaete sp. 2	14 ± 4	(2)	0.4 ± 0.2	(1)	**
Nauplii	925 ± 173	(36)	1316 ± 174	(21)	**
Rotifers	332 ± 94	(0)	817 ± 192	(0)	NS
Polychaete sp. 3	13 ± 2	(1)	43 ± 5	(0)	**
Spionid larvae	6081 ± 987	(210)	12684 ± 2687	(0)	NS
Veligers	1189 ± 256	(30)	1968 ± 141	(0)	**
Tanaids	0	(6)	0	(2)	
Caprellid amphipods	0	(10)	0	(4)	

Figure 3. Schematic pathway of an extended swimming bout exhibited by *Polyorchis* during observation periods. Position (1) shows an individual at rest before the bout with its tentacles extended in the sink-fishing posture. A swimming bout spontaneously begins with tentacles fully contracted (2). Asymmetrical velar contractions (3-5) cause the individual to swim in an arc until it is swimming directly downward (6). This downward swimming spontaneously stops (7), tentacles begin to relax as the bell returns to its original orientation (8). Tentacles are fully relaxed while the fishing posture and low frequency, maintenance swimming is resumed (9). V = velum.

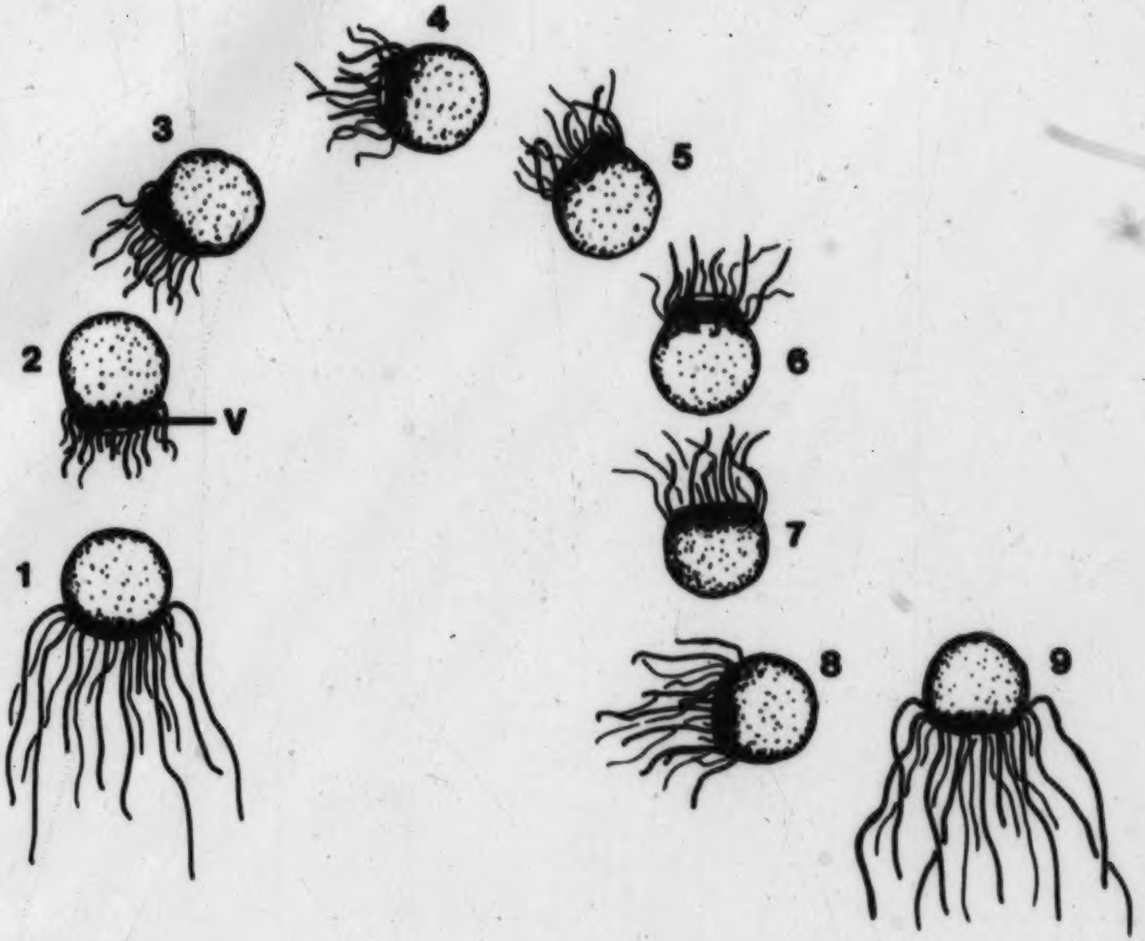
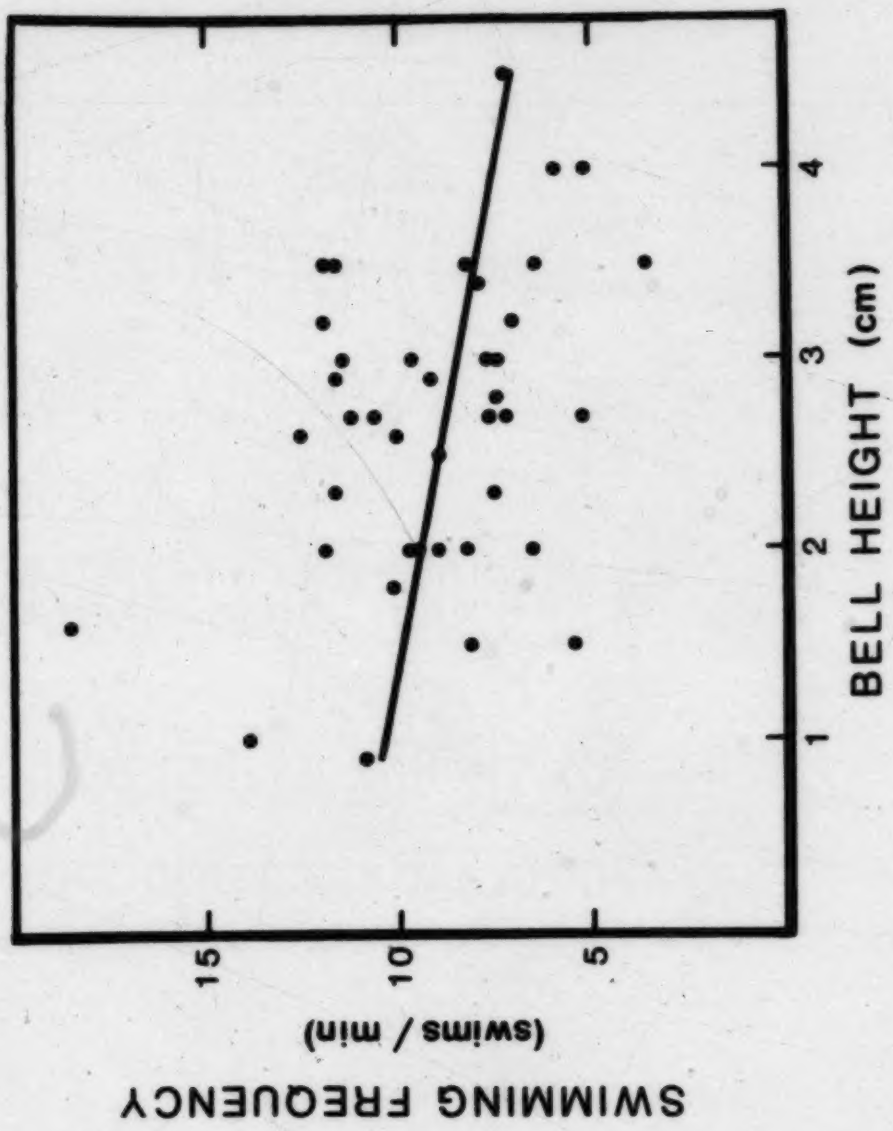

V

Figure 4. Swimming frequencies of 39 various sized individuals of *Polyorchis* during "maintenance swimming". Frequency was averaged over ten minute field observation periods. $Y = 11.28 - 0.97X$; $r = 0.323$ Regression is significant at $0.05>p>0.01$.


SWIMMING FREQUENCY
(swims / min)
15
10
5
BELL HEIGHT (cm)
1
2
3
4

during these brief swims. Local currents sometimes moved and entangled tentacles over the bell, or tilted the bell off vertical orientation. These disorientations did not cause extensive tentacle contractions, or changes in swimming frequency. Animals continued to swim at low frequencies while, owing to its center of gravity relative to its center of buoyancy, the bell righted passively.

Prey capture also disrupted the sink-fishing position of the tentacles. When small prey contacted tentacles, only tentacles involved in handling the prey contracted while the remaining tentacles held their fishing posture. The manubrium then made "searches" of the bell margin and made the transfer of prey from tentacles to manubrium. Only when large prey such as caprellid or gammarid amphipods came in contact with the tentacles did more tentacles become involved. This sometimes resulted in cessation of swimming and "crumpling" with subsequent sinking.

Maintenance swimming was occasionally interrupted by apparently spontaneous extended swimming bouts. Thirty percent of the individuals observed showed 1 to 4 separate extended swimming bouts during the 10 min. periods. These extended swimming bouts consisted of 10-20 swimming contractions at a frequency much higher than that of maintenance swimming. Individuals swam at a rate of between 1-2 swimming contractions per second depending on the size of the individual with smaller medusae swimming at greater frequencies than larger individuals. In these extended swimming bouts (Fig. 3), the first few contractions were usually symmetrical and only moved the individual several centimeters. After several contractions, obvious asymmetrical velar contractions caused individuals to turn in an arc with a diameter of about 20-30 cm, finally resulting in downward swimming. At this point, contractions ceased spontaneously, the bell righted to the original orientation, tentacles relaxed, and maintenance swimming resumed in the sink-fishing posture. This extended swimming bout did not usually result in a net vertical displacement, but it did move the medusa laterally. Variations on this general pattern were also observed. Some individuals did not make the downward turn, but continued to swim vertically. A continuation of downward swimming was also observed, often to the point where they were swimming against the substratum. Individuals also sat on their tentacles directly on substrata while the manubrium made "searches" around the bell margin and across the substratum.

Discussion

It is clear that *Polyorchis penicillatus* undergoes a small amplitude diel vertical migration within several meters of the bottom with an upward movement begining after sunset and lasting throughout the night. Just after sunrise, *Polyorchis* is down near the bottom where it remains throughout the day. This type of migration would have been missed by conventional sampling methods. Diel vertical migration of medusae has been documented most often by remote collection gear such as plankton nets, which sample robust species migrating large distances. However, species with small amplitude migrations, such as *Polyorchis*, may be missed or considered nonmigratory due to the poor depth resolution of plankton tows. Demersal species, may also be missed or damaged due to net-fouling by sediment or obstructions on the bottom. Direct observations of plankton through the use of SCUBA (Hamner et al. 1975; Omori and Hamner 1982) and submersibles (Mackie and Mills 1983) have eliminated some of these problems. Yet, some problems with direct observation of zooplankton distributions remain. A large number of sample replicates is often required to establish the distributional patterns amongst low density individuals, but replication is costly and time consuming and these considerations precluded the detection of the precise timing of vertical movements in my study. The improved temporal resolution of movements in Mills' (1983) tank study, however, showed that *Polyorchis* moves upward just after sunset and downward just after sunrise.

Data from emergence trapping show the typical diel activity pattern of most plankters with increased nighttime activity of several groups of large, mobile demersal plankters. Their marked absence or reduced numbers during daylight hours indicate that these groups either remain in or on the sediments or make only very short forays in the water column. The timing of upward and downward movements was not determined, but Alldredge and King (1980) found most polychaetes and gammarid amphipods emerged just after dusk and returned to sediments throughout the night. They also found that cumaceans emerged just after dusk, but returned to the bottom during dawn. Larval or juvenile stages of invertebrates consistently showed increased activity during the daytime. The sampling design of the traps precluded the determination of the extent of their movements, but most marine invertebrate larvae are photopositive during their early pelagic stages and this characteristic allows them to concentrate in the surface waters during the day (Thorson 1964). Two groups of demersal plankters known to be present in Bamfield Inlet but were conspicuously absent from trap samples were caprellid amphipods and tanaids. There are two possible reasons for their absence. Individuals may avoid capture and return to sediments when they contact the walls of the traps. This "wall effect" may then depress the number of individuals collected. Another possibility is that individuals from these two groups may make only very short forays away from the bottom. Even if they do in fact become more active at night, they may not reach the opening of the catch bottles. However, for most large, mobile taxa (e.g., gammarid amphipods, cumaceans, and polychaetes), it is clear that daytime movements are restricted to less than 0.5 m from the bottom, while nighttime movement is at least this far and probably farther.

The primary adaptive advantage of the diel vertical migration of ***Polyorchis*** is probably the optimization of prey encounters by matching movements with its principal prey, demersal plankton.. The positional shift, albeit of small amplitude, is sufficient to allow two different feeding modes and may permit prey selectivity. Clearly the planktonic sink-fishing behavior enables ***Polyorchis*** to capture items swimming in the water column. Sink-fishing is probably most advantageous during the night when most of the demersal plankton is moving, but this behavior yields to a greater dependence on substratum feeding during the day. The bottom-sitting behavior of ***Polyorchis*** has been observed before and is referred to as "landing" (Zelickman 1976) or "perching" (Mills 1981). Perching may be seen at anytime, but it is most important during the day when many of the prey are very close to the bottom or clinging to various substrata. Feeding close to or directly from substrata explains the presence within food boli of prey items (e.g., caprellid amphipods, tanaids) that are either nonmigratory or live close to or on substrata. This feeding mode may, however, not normally be as efficient as sink-fishing, except when ***Polyorchis*** lands on ***Agarum*** "islands" or other debris. The mechanism for the shifts from planktonic sink-fishing to perching or extended swimming bouts remains enigmatic. Many medusae go through cycles of some form of sink-fishing interspersed with active swimming (Zelickman 1976; Mackie 1980; Mills 1981). A possible explanation of these shifts is that individuals can detect prey densities by the frequency of encounters. If they enter areas of low encounters, they may make a "decision" to move and this is seen as an extended swimming bout. The large aggregations of medusae observed near *Agarum* "islands" also suggest that *Polyorchis* will remain in areas where prey density is high.

Study of the contents of food boli indicates that there may be some differential selection and utilization of prey items. Gammarid amphipods and cumaceans may be selectively captured as in some cases greater numbers of individuals were found in food boli than in trap samples. Larval or juvenile stages appear to be of minor importance as prey because few were found in food boli relative to the number collected in traps. Larval defences such as veliger shells or setae of spionid larvae may deter capture if nematocysts can not penetrate shells or extend past setae. Larvae may also escape predation by moving into surface waters during the daytime, thereby avoiding high concentrations of *Polyorchis* near the bottom. Most medusae may also not be able to effectively sink-fish in surface waters because turbulence may disrupt tentacle position and cause "crumpling" and sinking. Even though larval stages are occasionally captured, they may not be digested by *Polyorchis*. Many of the spionid larvae, veligers, and cyprids, though occasionally taken in large numbers, were found intact even at the proximal end of the bolus.

"Energetic" models have been invoked to explain the adaptive value of migrations, but they have been largely restricted to grazing plankters (Enright 1977). Medusae clearly are not under the same constraints as grazers, but may migrate to meet their energetic requirements. These energetic models have rivaled the "predator avoidance theory" (Zaret and Suffern 1976). Highly mobile and visible plankters, such as many demersal species, avoid predation through seclusive behavior during the daytime. These individuals then emerge to feed, spawn, or disperse during the night when the probability of detection is less. *Polyorchis* is probably visible and vulnerable to predation at any of its daytime positions in the water column. Nematocyst-laden tentacles may, however, deter predators, such as fish, and allow diel movements with impunity.

In addition to optimizing prey encounters, the diel migration of *Polyorchis* may also afford some advantage by synchronizing spawning locations. Mills (1983) found that *Polyorchis penicillatus* spawns in the hour immediately after dark and that spawning is complete within ten minutes. Concurrent movements of individuals together with very strong species specificity of sperm attraction (Miller 1980) may ensure a high percentage of fertilization. Nighttime movement away from the flocculent substratum may also be important in preventing gamete loss.

The unusual demersal feeding behavior of *Polyorchis* described in this study illustrates one example of the wide variety of behaviors used by predacious zooplankton to concentrate and capture prey. The planktonic sink-fishing posture of *Polyorchis* may serve as a model for explaining how most planktonic medusae optimize prey capture. Gerritsen and Strickler (1977) and Gerritsen (1980) have suggested several factors that affect the probability of contacting prey; swimming speed and direction, encounter radius, and prey density. All but prey density are behavioral factors and are controlled by the individual predator. Medusae use various combinations of these factors to increase their prey encounters (Fraser 1969; Mills 1981; Bailey and Batty 1983). The demersal feeding behavior of *Polyorchis* illustrates how these factors may be used by medusae for specialized feeding on demersal plankton. Gerritsen and Strickler's model predicts two energetically optimal swimming strategies for invertebrate zooplankton predators; "ambush" and "cruising". *Polyorchis* and most medusae are typical ambush predators; those which move slowly relative to their prey items. *Polyorchis* remains nearly motionless in the water column except for the low frequency maintenance swimming and slow sinking. This movement is very slow relative to principal prey items, the large, fast-moving demersal plankters (e.g., cumaceans, gammarid amphipods). Gerritsen (1980) also predicted that the highest encounter rate results when predator and prey items swim perpendicular to each other. For a sink-fishing medusa like *Polyorchis*, the sinking is slow enough that there may be little advantage gained from this, however, the tentacle arrangement while sink-fishing may increase encounter rates by its orthogonality to prey movements.

Tentacle arrangement during sink-fishing may increase encounter rates by altering the encounter radius. Encounter radius, as defined by Gerritsen and Strickler (1977), is a composite of various sensory systems, habitat conditions, and prey type, but for sink-fishing predators like *Polyorchis* , it may be simplified to the area of tentacle extension (Figure 3). The cylindrical arrangement of tentacles consists of both horizontal and vertical components. These components provide orthogonal placement of tentacles and capture prey items moving vertically and horizontally. A large individual with a bell height of 4.0 cm in a typical sink-fishing posture can fully extend its tentacles 12-15 cm downward and held outward in a 9-11 cm diameter circle. This posture can effectively capture all prey items in a cylindrical area of 750-1500 cm^3. If individuals become aggregated in large numbers, they may fish out large volumes of water. These large aggregations may be influential in reducing larval fish stocks and other plankters (Plotnikova 1961; Sveshnikov 1963; Zelickman 1969; Bailey and Batty 1983).

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III. TREADMILL EXPERIMENTS

Photoresponses of a hydromedusan on a "treadmill": possible behavioral mechanisms of diel vertical migration

Introduction

Many hydromedusae show distinct behavioral responses to changing light conditions. Some respond to rapidly changing light intensities by a behavior that has been generally referred to as an "off response" (Singla 1974) or a "shadow response" (Tamasige and Yamaguchi 1967). This behavior consists of a few rapid swimming contractions following rapid reductions in light intensity. Although the function of this well-defined behavior has never been demonstrated, it has by convention been considered a predator avoidance mechanism. Many hydromedusae also show distinct responses to light intensity changes as evidenced by their diel vertical migrations (Russell 1925; Moreira 1973; Mills 1982). A few, such as *Bougainvillia*, *Gonionemus*, *Polyorchis*, *Spirocodon*, and *Stomotoca* that have been shown to have some kind of shadow response (Murbach 1909; Hisada 1956; Singla 1974; Mackie 1975; Anderson and Mackie 1977) also make distinct diel vertical migrations (Kikcuchi 1947; Mills 1982,1983). Even though rapidly changing light intensity at dusk and dawn is generally considered one of the most important cues used by zooplankters in regulating diel vertical migration (Forward 1976a), the functional relationship between the shadow response of hydromedusae and their diel migration has not been previously considered. The purpose of this study was to characterize the photic responses of various sizes, and thus ages, of the hydromedusan *Polyorchis penicillatus* and to suggest roles for these responses in the regulation of its diel movements.

Several laboratory studies have attempted to look at the photic behavior of medusae (Murbach 1909; Mackie et al. 1981; Mills 1983), but these studies have been done with medusae swimming freely in tanks. There are at least two problems with these studies which may bias their results. First, medusae confined in a tank often collide with its walls and this may cause a change in swimming activity. Swimming may be either inhibited by "crumpling" or increased by excitation of the tentacles when they contact tank walls. Secondly, with free-swimming medusae, it is difficult to control the lighting regime since the position of the medusa in the water column and the orientation of its ocelli with regard to light sources is constantly changing. I have eliminated these problems in this study by devising a treadmill that allows tethered swimming of medusae without "wall effects" and also maintains a constant orientation of ocelli to the controllable lighting.

Swimming frequency was found to be directly proportional to the rate of decrease in light intensity. Slowly increasing light intensity caused an inhibition of swimming. In addition, rapid 100% shadows of varying absolute magnitude usually produced only a single swimming contraction. These results suggest that the shadow response of *Polyorchis* does not function in predator avoidance, but is more likely to contribute to the nighttime upward movement. Differences in the photic responses of various size classes suggest ontogenetic changes in the photic behavior of *Polyorchis*. These changes may contribute to distributional differences in the field with small (young) individuals staying in the surface waters and larger (mature) individuals assuming a more demersal existence.

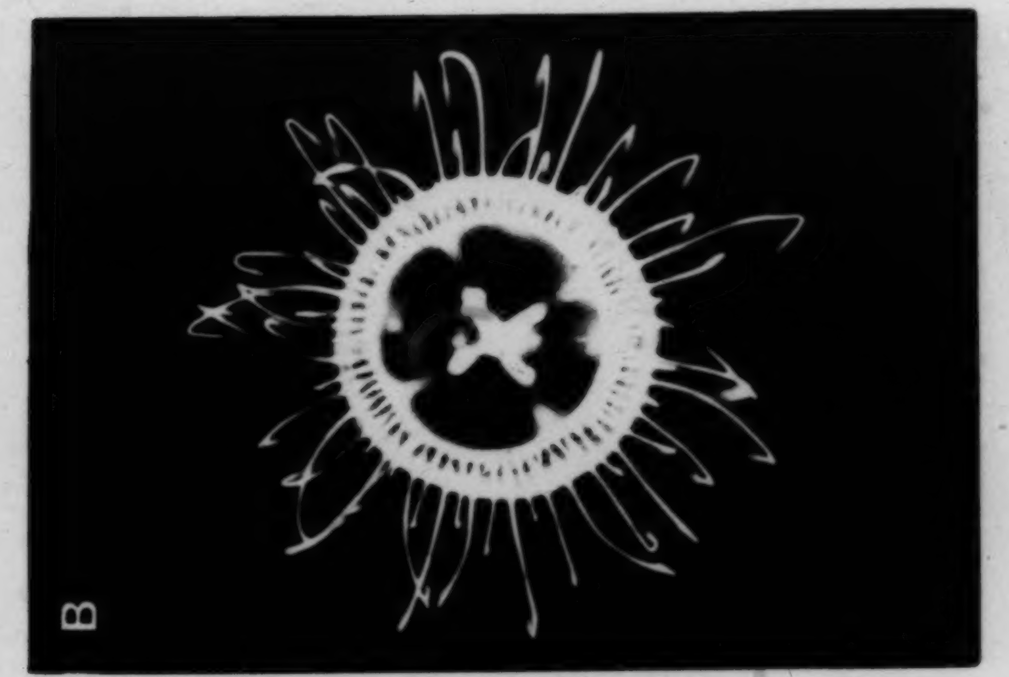
Methods and Materials

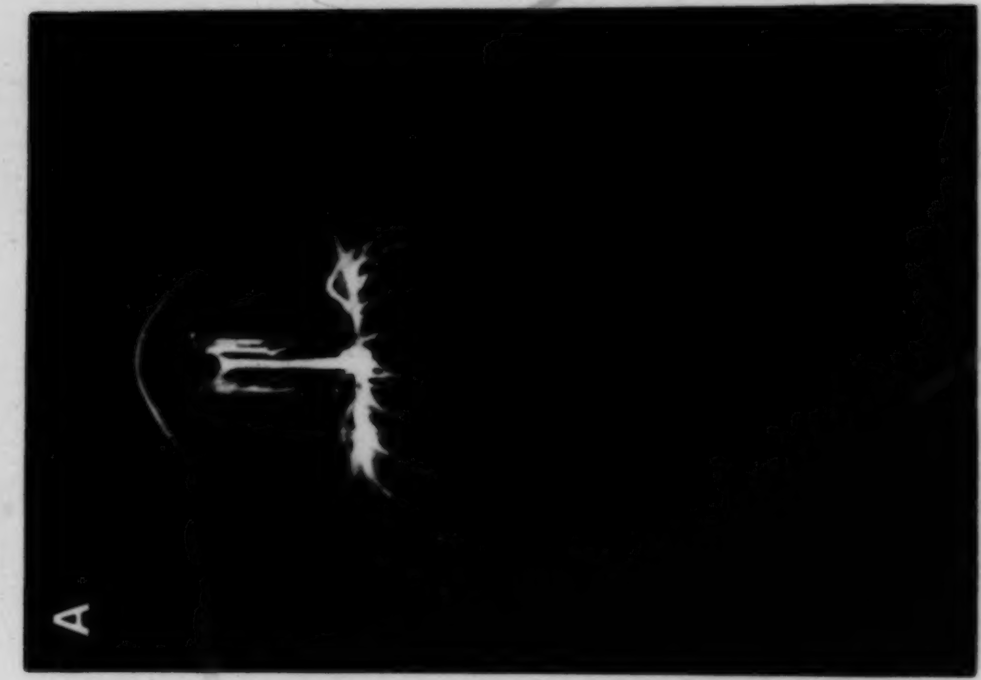
Description of treadmill

Figure 5 shows an individual medusa suspended on the treadmill. Various sizes of individuals (bell height measured from apex of bell to bell margin) were suspended on the treadmill by fine (1mm diameter) glass tubing. Some very small individuals required suspension by finer wire. Tubing pierced the exumbrellar epithelial layer of the bell and passed horizontally through the thick mesogloea at the bell apex. Neither swimming musculature on the subumbrellar surface nor any nervous tissue was disrupted. Tubing ends rested on the sides of a 21x21x18 cm plexiglass tank and thus maintained the bell margin bearing the ocelli horizontal as well as prevented the tentacles from touching the sides and bottom of the tank. In this position, individuals could freely perform all of their behavior (e.g., swimming, crumpling, feeding) without any mechanical restrictions. Seawater temperature in the treadmill ranged from 10-13 °C. Treadmill seawater was changed after each individual medusa. Individuals of the hydromedusan *Polyorchis penicillatus* used in this study were collected by divers from Bamfield Inlet and Pachena Bay, Bamfield, B.C. Medusae were kept unfed in running seawater and were used within 3-4 days. All animals were kept under natural photoperiod prior to the experiments.

The light source for these experiments was a Volpi AG Intralux fiber optic system equipped with a halogen lamp. Absolute light intensities were measured with a Licor LI-185 Quantum Sensor. Light intensities were altered by opening and closing an iris diaphragm located between the light source and fiber optic and by adjusting distances between the fiber optic head and bell margin. Specifics of lighting conditions for each experiment are given in their respective sections. All experiments were conducted in a darkened room. The light intensity in the room was below that detectable by the Licor light meter (less than 0.1 microeinsteins / m^2 s). Thus, percentage changes in light intensity were based on decreases or increases in light intensity from the light source relative to the light intensity of the darkened room.

Figure 5. Side (a) and aboral (b) view of ***Polyorchis*** suspended on the treadmill. Medusae were completely immersed and free from contact with sides and bottom of the tank. Individuals often assumed this typical "sink-fishing" posture shown here and could swim freely. Fiber optic lighting was suspended directly above the individual. Distances between the marks on glass tubing in (a) is 1 cm.


B


A

Medusa size / swimming frequency during extended swimming bouts

Seventeen individuals of various sizes were placed one at a time on the treadmill and allowed to acclimate in room light for 15 minutes. The total number of swimming contractions during spontaneous extended swimming bouts were counted. Three to five separate bouts were observed for each individual. The mean number of swimming contractions per minute was plotted as a function of bell height. No special lighting conditions other than ceiling fluorescent lights were used in this experiment.

Swimming frequency in constant light

Twenty-nine individual medusae were placed on the treadmill and were allowed to acclimate for 15 minutes at LOW (9.7 microeinsteins/ m^2 s) light intensity. At the start of the experiment, the light intensity was increased (by opening the diaphram) over 15 seconds to the HIGH (280.7 microeinsteins/ m^2 s) light intensity. Swimming contractions were not counted during the light changing periods, but commenced at the end of the 15 s period. The number of swimming contractions were counted for a total of 15 minutes with the first 2 minutes divided into 15 s intervals. After 2 minutes, swimming contractions were summed over 1 minute intervals. At the end of the 15 minute trial period, the light intensity was decreased (by closing the diaphragm) over 15 s to the LOW light level and swimming contractions were counted as before. This procedure was followed by 1 more HIGH light and 1 more LOW light trial, resulting in two LOW and two HIGH light trials per individual. A two-way ANOVA (Sokal and Rohlf 1969) was performed on log $(x+1)$ transformed values (x=calculated number of swimming contractions per second) comparing swimming frequency at 2 light levels and 21 time periods. Log $(x+1)$ transform was used because the variances increased with increasing means.

Response to rapid 100% changes in light intensity

Nine individuals of various sizes were placed on the treadmill one at a time and allowed to acclimate in the dark for 15 minutes. This time period was found to be sufficient for medusae to relax tentacles and maintain a sink fishing posture. Four light intensities (280.7, 65.8, 9.7, 2.4 microeinsteins / m^2 s) were presented separately for 2 minutes and then a rapid OFF-ON (approximately 0.5 s) 100% shadow was made by passing a card between the fiber optic head and the individual. The percent shadow was determined by $(I_0 - I_1) / I_0$ where I_0 = initial absolute light intensity and I_1 = light intensity after 0.5 s (in this case approaching 0 microeinsteins / m^2 s). During the two minute pre-shadow period, the number of swimming contractions per minute was counted. After the shadow, the number of swimming contractions in five seconds was recorded. The shadow response, as indicated by the number of swimming contractions per minute following the stimulus, was determined by subtracting the pre-shadow swimming frequency from the post-shadow swimming frequency. Four trials per individual were made at each of the four light intensities. Comparisons of the mean swimming frequency (after log (x+1) transform) of the shadow response at four different absolute light intensities were made by a one-way ANOVA (Sokal and Rohlf 1969).

Spectral sensitivity of the shadow response

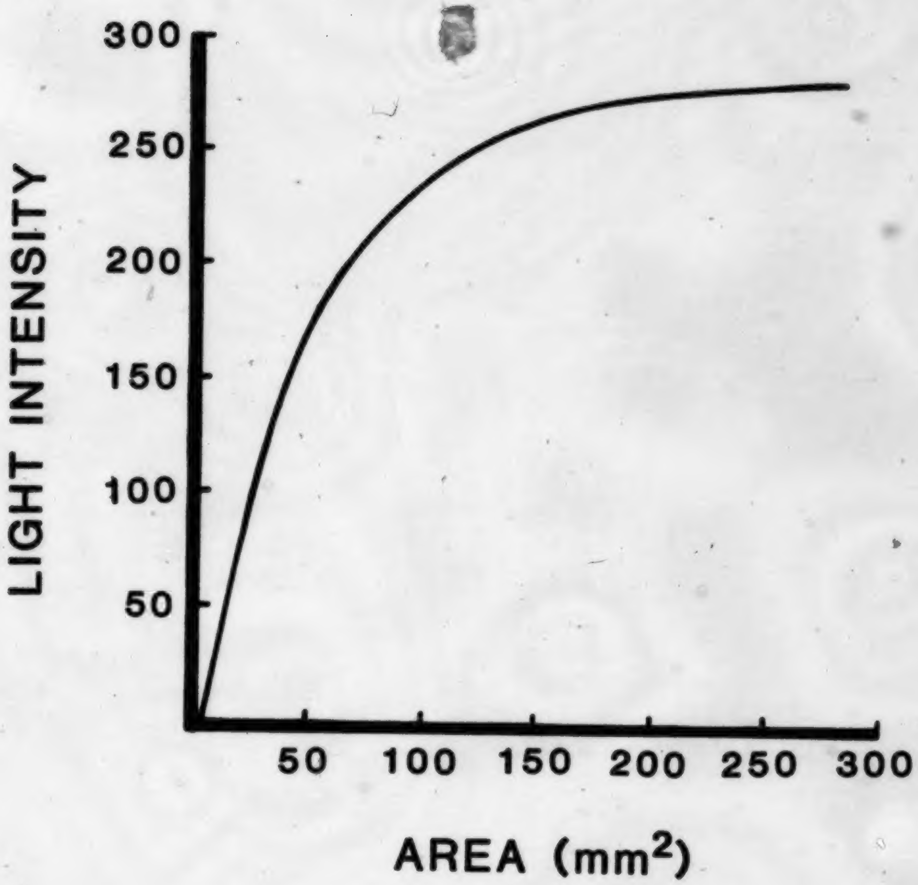
The spectral sensitivity of the shadow response was determined by presenting rapid shadows of monochromatic light. Seventeen various sized individuals were placed on the treadmill and were allowed to acclimate in the dark for 15 minutes. They were then exposed to wavelengths of monochromatic light ranging from 425-700 nm in 25 nm increments. Monochromatic light was produced by shining the fiber optic light source into a Bausch and Lomb Monochrometer (Band pass width 19.2 µm; dispersion 6.4 µm/mm; first order range 350-800 nm). All light levels were adjusted to 0.7 microeinsteins/m^2 s. This was the greatest light intensity attainable for all wavelengths. Medusae were presented with monochromatic light for 2 minutes and then given a 0.5 s, 100% shadow. If a swimming contraction followed the shadow within 1 s, I considered that the individual responded to that wavelength and assigned a value of 1 to that trial. Additional swimming contractions after 1s were judged to be not directly due to the shadow and were not counted. If it did not respond, I assigned a value of 0. Four such trials for each individual were made at each wavelength increment. Spectral sensitivity is reported as a percent of the maximum possible number of responses.

Swimming response to continuous changes in light intensity

Eleven medusae of various sizes were again placed on the treadmill and allowed to acclimate in the dark for 15 minutes. Individuals were then given four rates of light intensity change and the observed swimming frequency was recorded. Changing light conditions were produced by manually opening and closing an iris diaphragm located between the fiber optic and the light source. The light intensity at the bell margin ranged from 280.7 microeinsteins / m^2 s (diaphragm fully open, HIGH) to 9.7 microeinsteins / m^2 s (LOW). The light intensity changes produced by opening and closing the diaphragm were not linear but followed a curve shown in Figure 6. Changes in diaphragm position (HIGH to LOW and LOW to HIGH) were made over four time periods (1, 15, 30, 60 s) and corresponded to the log of the mean percent change in light intensity per s ($\Delta I_{\bar{x}}$) of -0.015, -0.914, -1.123, -1.344, respectively.

Individuals were exposed to LOW light for 15 minutes after which the diaphragm was opened to HIGH, followed by a change from HIGH to LOW, then LOW to HIGH,and HIGH to LOW. A 2 minute interval separated each change and 5 such increasing and 5 decreasing light intensity trials were given to each individual for four rates of change. The total number of swimming contractions during these periods of increasing and decreasing light were counted and the mean number of swims per minute was calculated. Comparisons between the mean swimming frequency at each rate of light change were made by one-way ANOVA (Sokal and Rohlf 1969).

Figure 6. Plot of the measured area of iris diaphragm aperture and measured light intensity (microeinsteins / m^2 s) of the fiber optic at a distance of 10 cm. The curve was divided into 1, 15, 30, 60 second intervals and the absolute light intensity change per s was calculated for each of the four time intervals. The mean rate of percent change in light intensity per s ($\Delta I_{\bar{x}}$) was determined by $(I_0 - I_1) / I_0$ where I_0 = initial absolute light intensity and I_1 is the absolute light intensity after one second.


300
250
200
150
100
50
LIGHT INTENSITY
50
100
150
200
250
300
AREA (mm²)

Results

Medusa size / swimming frequency during extended swimming bouts

Individual swimming bout durations ranged from as short as fifteen seconds to longer than five minutes. Swimming frequency during each bout was usually very regular. Quiescent periods of "sink-fishing" or an occasional "crumple" separated bouts. The mean swimming frequency of *Polyorchis* during extended swimming bouts increased exponentially with decreasing bell height (Fig. 7).

Swimming frequency in constant light

Smaller medusae swam at a greater frequency under constant HIGH light intensity than under LOW light intensity conditions; larger medusae swam at nearly the same frequency regardless of the light condition (Fig. 8). The total number of individuals were separated into three arbitrary size classes, those with bell heights less than 1.0 cm, 1-2 cm, and those greater than 2.0 cm. Two-way ANOVA (Sokal and Rohlf 1969) comparing the mean number of swimming contractions per second at two light intensities and 21 time increments showed that medusae with bell heights less than 1.0 cm swam at significantly ($p < 0.001$) greater frequency under HIGH than under LOW light intensity. For the two groups of medusae with bell heights greater than 1.0 cm, there was no significant ($p > 0.05$) difference in swimming frequency under the two light conditions. Although the group of smallest medusae showed an initial reduction in swimming frequency, suggesting some adaptation to the light intensity, none of the groups showed a significant ($p > 0.05$) difference in swimming frequency over the 15 minute period.

Responses to rapid 100% changes in light intensity

Medusae usually responded to rapid 100% shadows with 1 rapid swimming contraction, regardless of the absolute magnitude of the light intensity change (Table II). During the two minute pre-shadow period, most of the individuals did not swim and

Figure 7. Mean (±1SE) number of swimming contractions per minute of various sizes of *Polyorchis* observed during extended spontaneous swimming bouts on the treadmill. An exponential curve ($Y = 132.65\ e^{-0.93x}$; $r=0.96$) has been fitted to the mean swimming frequencies. The smallest individuals used were early eight and sixteen tentacle stages.

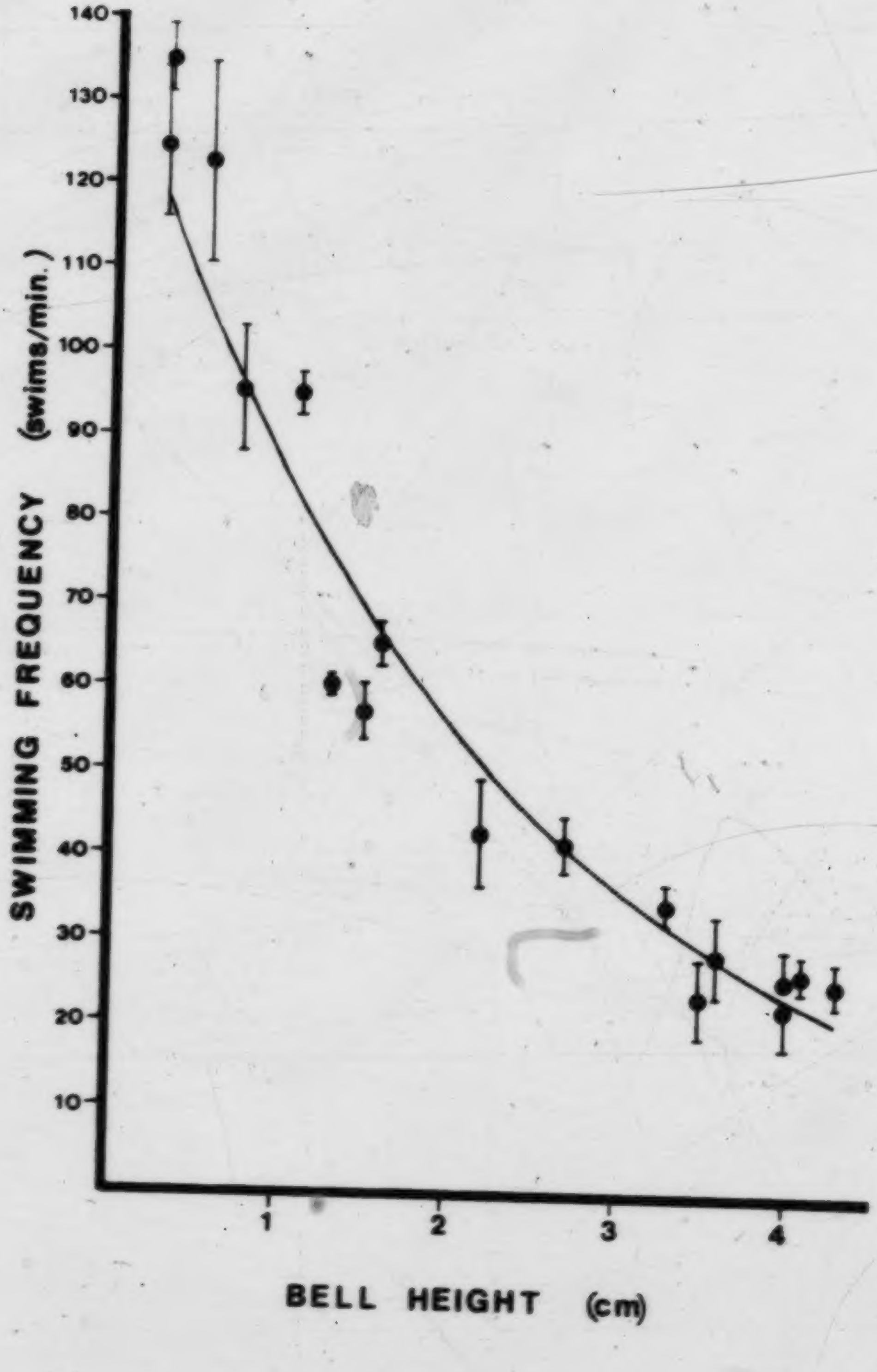
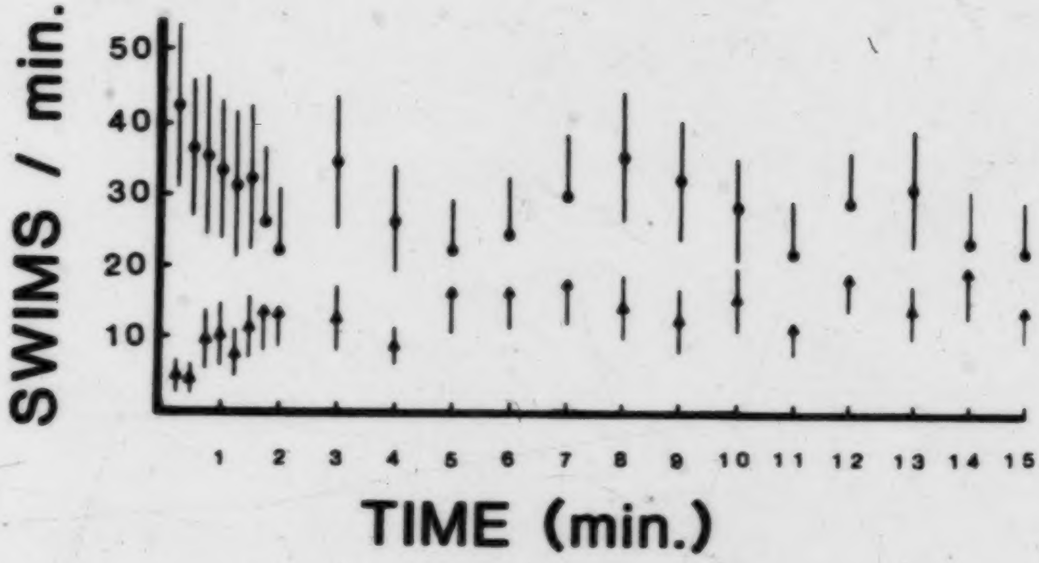

SWIMMING FREQUENCY (swims/min.)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
1
2
3
4
BELL HEIGHT (cm)

Figure 8. Mean (±1SE) number of swimming contractions per minute by *Polyorchis* on the treadmill over 15 min periods at HIGH (● 280.7 microeinsteins / m^2 s) and LOW (▲ 9.7 microeinsteins / m^2 s) light intensity levels. Swimming frequency of individuals with bell heights less than 1.0 cm (range 0.3-0.7 cm, n=24) (top); Swimming frequency of individuals with bell heights between 1-2 cm (range 1.2-1.8 cm, n=16) (middle); Swimming frequency of individuals with bell heights greater than 2.0 cm (range 2.1-3.4 cm, n=18) (bottom). Notice the size class differences in overall swimming frequency with smaller medusae swimming at a much higher frequency than larger individuals. N.B., the means and SE presented here are from raw data and are *not* antilogarithms of the log (x+1) transformed data.


SWIMS / min.
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
TIME (min.)

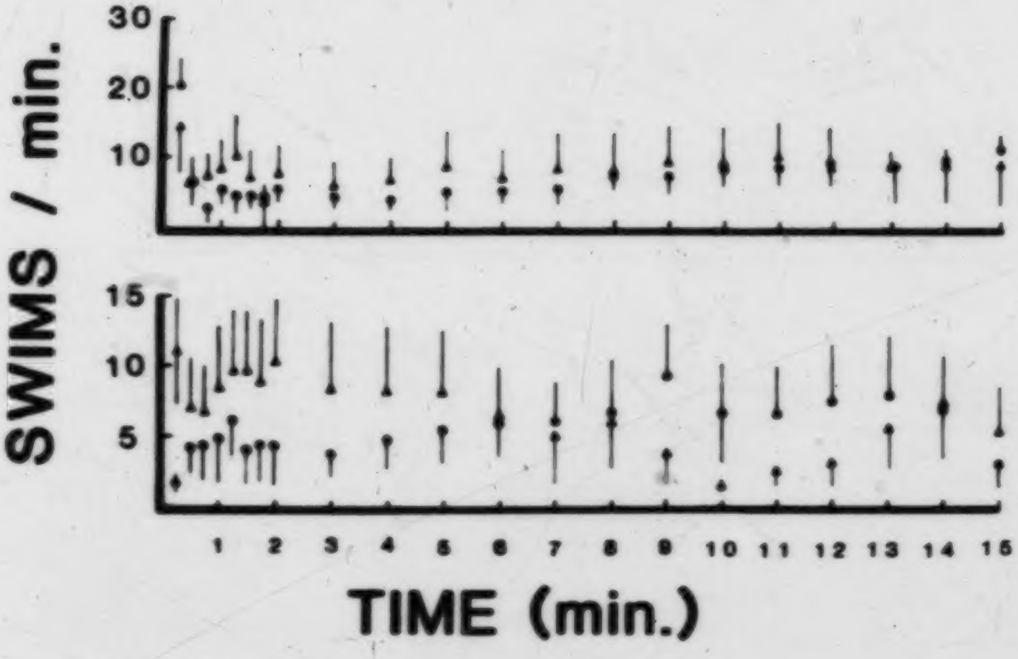

SWIMS / min.
30
20
10
15
10
5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
TIME (min.)

Table II. Mean (±1 SE) number of swimming contractions in the 5s period after a rapid 100% shadow of four different absolute light intensity changes. Comparisons of the mean shadow response frequency at four absolute light changes by one-way ANOVA showed no significant ($p > 0.05$) difference in the responses.

Light Intensity (microeinsteins / m^2 s)			
2.4	9.2	65.8	280.7
0.84 (0.08)	1.08 (0.09)	1.01 (0.13)	0.98 (0.02)

Table III. Mean (±1 SE) total number of swimming contractions during four different rates of percentage change in light intensity ($\Delta I_{\bar{x}}$) Values are for decreasing light intensity only. 1) For individuals with bell heights less than 2.0 cm, n=30. 2) For individuals with bell heights greater than 2.0 cm, n=25.

	LOG $\Delta I_{\bar{x}}$			
	-0.015	-0.914	-1.123	-1.344
1	1.51 (0.12)	12.73 (1.17)	15.53 (2.02)	12.17 (2.83)
2	1.22 (0.03)	13.08 (0.52)	21.00 (1.03)	28.76 (2.73)

remained motionless in the sink-fishing posture. Immediately after the brief (0.5s) shadow was presented, nearly all of these individuals responded with one swimming contraction. This single swim did not lead to extended swimming bouts and the medusae returned to their sink-fishing posture after the swim. For those medusae that were swimming during the pre-shadow period, the response to the shadow was 1 interpolated swimming contraction in its pre-shadow swimming frequency.

Spectral sensitivity of the shadow response

All medusae consistently responded to shadows of monochromatic light between 450-550 nm (Fig. 9). Small medusae responded to a broad range with a peak response at 450 nm while the response range of larger medusae was slightly compressed and the peak response shifted to slightly longer (550 nm) wavelength. This peak and spectral distribution of the shadow response is similar to the spectral response of the electroretinogram (ERG) of *Polyorchis* found by Weber (1982). Smaller individuals were generally more responsive to all wavelengths as indicated by the overall higher percentage of responses.

Swimming response to continuous changes in light intensity

The swimming frequency of *Polyorchis* decreased markedly as the rate of percentage light intensity change decreased (Fig. 10). Swimming usually only lasted as long as the shadow period so that even though the highest swimming frequency was observed during the most rapid light reductions, the total number of swimming contractions in the response was small (Table III). The greatest total number of swims occurred during the slowest rate of percentage light change as medusae swam nearly continuously throughout the shadow periods. Smaller medusae showed a greater overall swimming frequency and were much more responsive to rapid shadows (i.e., exhibited greater swimming frequency than larger medusae). For slower shadows, however, larger medusae showed a greater swimming frequency.

During increasing light, medusae rarely swam and often "crumpled" resulting in marked differences in swimming frequencies from those seen during decreasing light intensity (Fig. 10). Crumpling behavior was most obvious during the slower light

Figure 9. Percent of the maximum possible number of shadow responses to 0.5 s shadow of monochromatic light. Solid columns indicate individuals with bell heights less than 2.0 cm (10 individuals), open columns indicate individuals with bell heights greater than 2.0 cm (7 individuals).

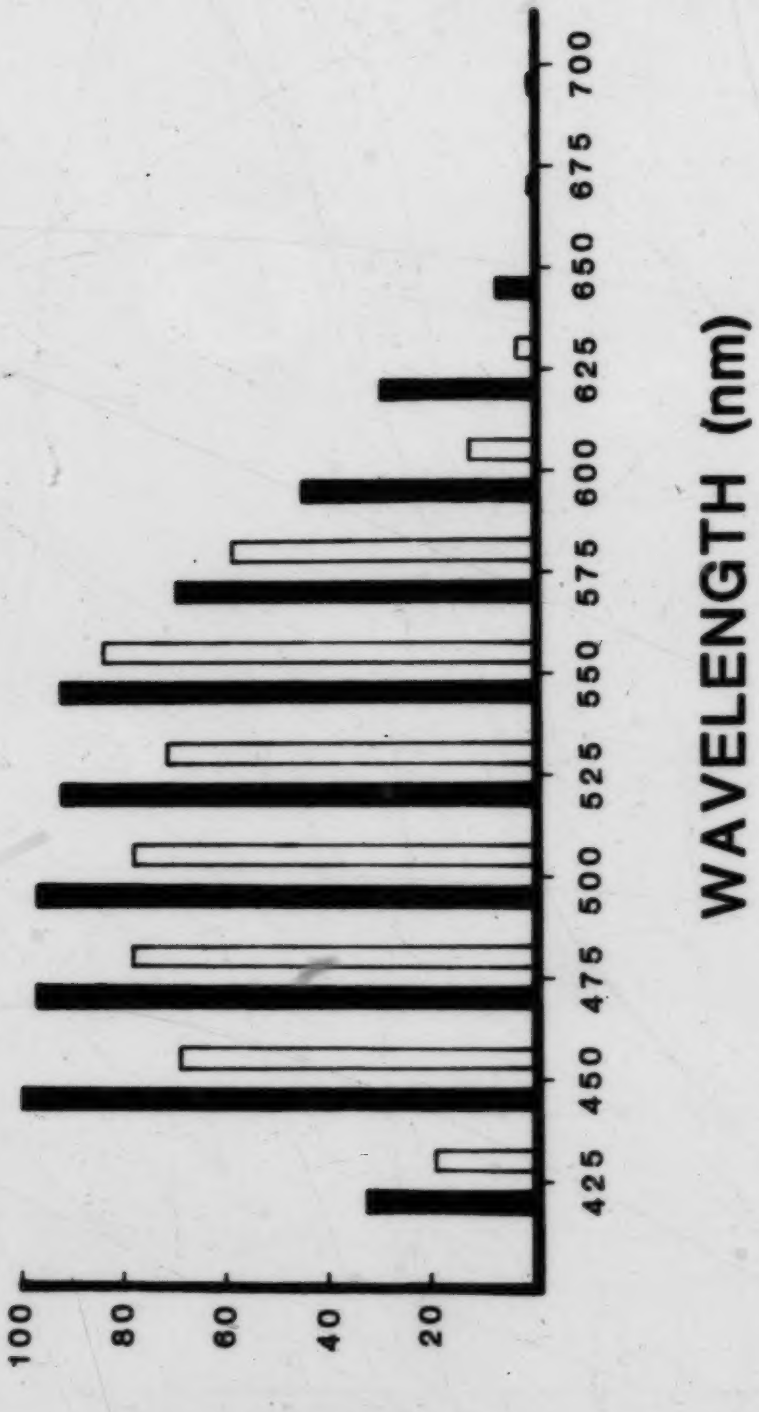
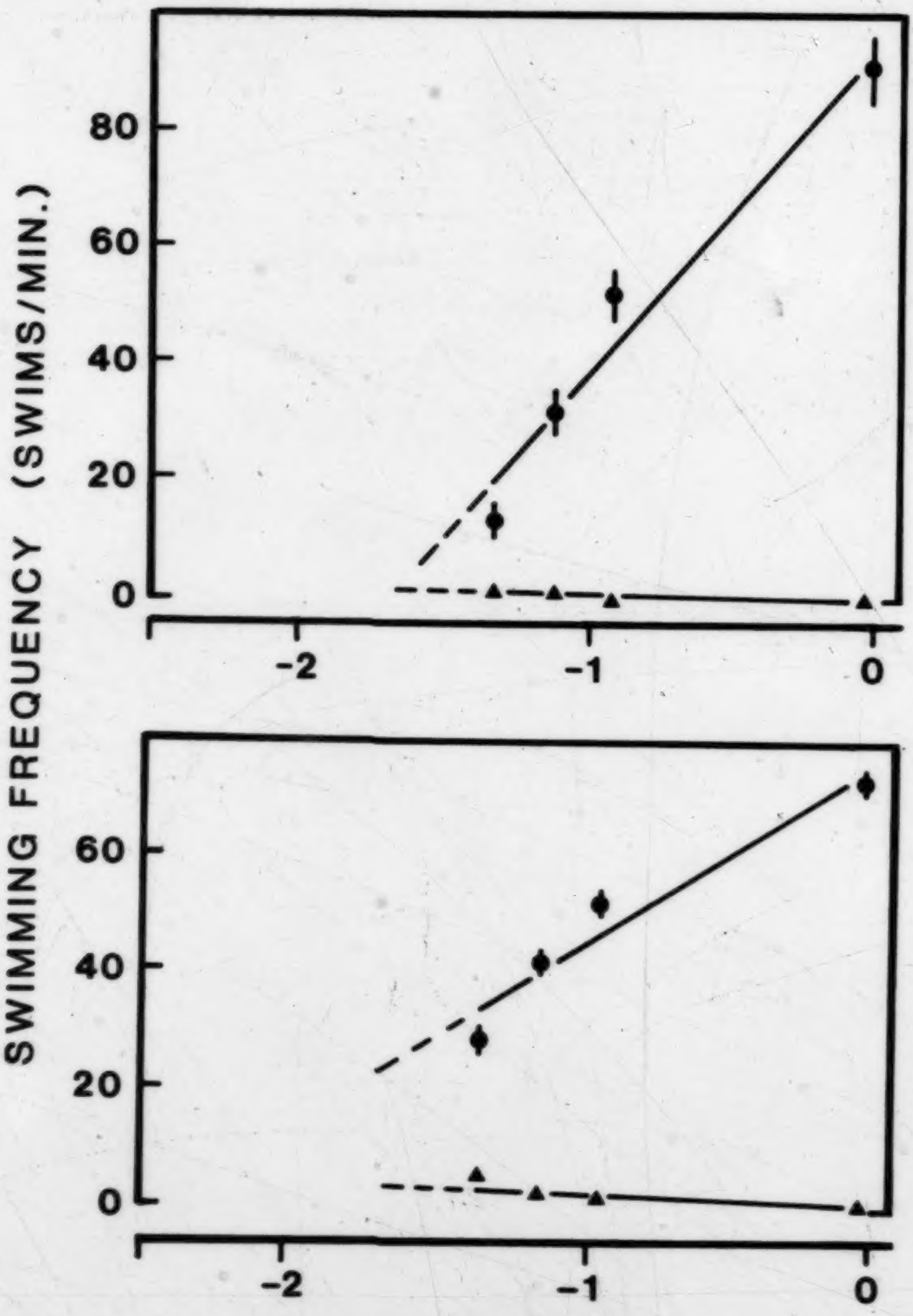

PERCENT
100
80
60
40
20
425
450
475
500
525
550
575
600
625
650
675
700
WAVELENGTH (nm)

Figure 10. Mean (±1SE) number of swimming contractions per minute by *Polyorchis* in response to various rates of percent change in light intensity per s ($\Delta I_{\bar{x}}$). Top figure shows the response of medusae with bell heights less than 2.0 cm (range 0.7-1.9 cm, 6 individuals). There was a significant ($p < 0.001$) difference between the four mean swimming frequencies and a significant ($0.05 > p > 0.01$) linear regression ($Y = 94.42 + 56.77 X$) for decreasing (●) light. No significant ($p > 0.05$) difference between the mean swimming frequencies or regression ($Y = -0.16 - 0.52 X$) for increasing (▲) light. Bottom figure shows the response of medusae with bell heights greater than 2.0 cm (range 2.4-2.8 cm, 5 individuals). There was a significant ($p < 0.001$) difference between the four mean swimming frequencies and a significant ($0.05 > p > 0.01$) linear regression ($Y = 75.40 + 31.09 X$) for decreasing (●) light. For increasing light (▲), swimming frequency at the slowest rate of change was significantly ($0.05 > p > 0.01$) different than swimming frequencies at the three faster rates. Linear regression ($Y = 0.43 - 2.49 X$) is not significant ($p > 0.05$). For both size classes, swimming frequencies at the greatest rate of percentage decrease is probably close to the maximum swimming frequency which may impart some curvilinearity to the function. Dotted lines show predicted swimming frequencies for other rates of light intensity changes.


SWIMMING FREQUENCY (SWIMS/MIN.)
80
60
40
20
0
-2
-1
0
60
40
20
0
-2
-1
0
LOG △ I$_{\bar{x}}$

intensity increases and consisted of progressive tentacle contractions, bell margin involutions, and radial muscle contractions. Once fully crumpled, individuals often remained so for several minutes. If a medusa was in the crumpled position at the start of a successive decreasing light intensity trial, it usually relaxed the severe radial and marginal contractions, responded to the shadow with a swimming contraction and began to swim at a frequency proportional to the rate of light intensity decrease. There were no significant differences in the response to increasing light intensity by the different sizes of *Polyorchis* (Fig. 10); nearly all individuals, regardless of size, showed at least a partial crumpling.

Discussion

A shadow response in some form has traditionally been thought to function as a predator avoidance mechanism (Coldstream 1836 in Gwilliam 1963; Gwilliam 1965; Forward 1976b; Forward 1977). By analogy, the shadow reponse, exhibited by some medusae, has also generally been considered to function in predator avoidance (Singla 1974; Anderson and Mackie 1977). However, results from this study indicate that for *Polyorchis*, this is an unlikely function of its pronounced response to reductions in light intensity. A typical shadow generated by some cruising predator during the daytime would be a rapid OFF-ON shadow of a duration similar to that used for the experiments shown in Table II, but they would likely be much less than 100% decreases of original light intensity. Even if the light intensity reduction was sufficient to produce a response, it is clear that *Polyorchis* would respond to such a shadow with only 1-2 additional swimming contractions (Table II). The small number of swimming contractions generated by this shadow would not propel the individual an effective escape distance, because distances travelled are negligible until the medusa has reached maximum velocity, usually after 1-2 contractions (Gladfelter 1972; Daniel 1983). Furthermore, the increase in light intensity after the shadow would inhibit any further swimming contractions (Fig. 10). Even if *Polyorchis* does respond to a predator-generated shadow, it seems unlikely that this movement would yield any advantage to the individual, but rather a disadvantage. Because the potential predator creating the shadow would necessarily be above the medusa, the upward swimming in response to such a shadow would only increase the probability of detection and capture as the medusa moves closer to the predator. If the shadow response exhibited by *Polyorchis* is not used for predator avoidance, what is the function of this well-developed behavior?

Correlations between the rate of light intensity change and the amplitude and velocity of vertical migrations of individuals or populations of zooplankters (primarily crustaceans) have been well documented (McNaught and Hasler 1964; Ringelberg 1964; Buchanan and Haney 1980; Stearns and Forward 1984). A similar relationship may exist for *Polyorchis* and other medusae which have a pronounced shadow response. In this study, changing light conditions have been shown to be important in causing the greatest changes in the swimming activity of the hydromedusan *Polyorchis*. If these changes are to result in net movements, however, responses to light stimuli must differ from the "normal" swimming frequency. Field observations have shown that *Polyorchis* spends most of its time swimming at a frequency of 5-15 swims per minute as it drifts in the water (Chapter 2; Arkett 1984). This behavior has been referred to as "maintenance swimming" and may be considered the "normal" swimming frequency for *Polyorchis*. Because net vertical movement during maintenance swimming is negligible, any increase or decrease in this swimming frequency over extended periods of time should result in a change of position in the water column. Swimming frequencies seen under the constant light experiments in Figure 8 are similar to those of "maintenance swimming" (Chapter 2). Clearly, constant light intensities of different absolute magnitude do not cause swimming frequency differences for larger medusae at least (but see below). *Polyorchis* responds to rapid 100% OFF-ON shadows of varying absolute magnitude with one quick swimming contraction (Table II), but this response does not alter the overall maintenance swimming frequency. Marked differences in maintenance swimming have been observed in the field during extended swimming bouts as *Polyorchis* usually swims in an arc at frequencies close to those seen in Figure 7. Even though the swimming frequency and the duration of these bouts are sufficient to displace the individual appreciable distances, these bouts are transient, apparently spontaneous, and are probably not a direct result of changing light conditions and hence can not explain diel movements. However, slow, continuous reductions in light intensity have been shown to produce nearly continuous swimming at frequencies above maintenance swimming and for extended periods (Fig. 10). These recurring shadow responses should displace individuals significant distances and result in upward movements.

Whether this phenomenon really does contribute to diel vertical migration of *Polyorchis* depends upon the rate of change in light intensity in field conditions. I attempted to measure the rate of light intensity change every 10 min. during sunset and sunrise in Bamfield Inlet on several different days. Typical absolute light intensities during these periods near the bottom were less than 10 microeinsteins / m^2 s. The greatest rate of change I could record before the light intensity fell below the sensivity of the light meter, was 90% in 10 minutes (=0.0015/s, log x=-2.82). This value is over 10 times slower than the slowest rate I could produce in the experiments shown in Fig. 10. However, this rate of light intensity decrease was recorded very close to actual sunset and in one of the few studies that recorded rates of change in light intensity, Munz and McFarland (1973) showed that the greatest rate of light intensity decrease does not occur until *after* sunset and for about 40 minutes after that time. During this period, they recorded rates of percentage decrease in light intensity (over a 10 min period) of approximately 0.0021 / s (log x = -2.67). Steans and Forward's (1984) study in estuarine coastal waters showed that the rate of percent change in light intensity (over a 10 min interval from between sunset and to about 50 min after sunset) was approximately 0.0016 / s (log x = -2.81). It is important to note here that these rates are integrated over 10 min intervals and the rate of percentage change calculated over 1 min intervals or less would very likely be faster. In any event, the predicted swimming frequency of large individuals (greater than 2.0 cm, Fig. 10) for these rates of change in light intensity is close to or slightly greater than those found during constant light intensity (Fig. 8) and the "maintenance swimming" frequencies found in the field (Fig. 4). This small increase in swimming frequency may then contribute to the initiation of upward movement at sunset and diel vertical migration. This explanation does not appear to hold for small medusae (less than 2.0 cm) as their predicted swimming frequency for rates of change in light intensity at sunset is well below that of their "maintenance swimming" frequency and their swimming frequency in constant light intensities (Chapter 2).

The threshold rate of light intensity decrease for the shadow response appears to ensure that upward movements would begin after sunset. Slower rates of light decrease, which occur late in the day and early evening, would not be likely to cause a shadow response. Even rapid shadows that occur during the daytime due to wave and surface turbulence would be ineffective because subsequent increases in light intensity would inhibit swimming. Swimming frequency would thus not differ from maintenance swimming and no appreciable upward movement would be observed. As dusk approaches, however, the rate of light intensity decrease is continuous and accelerates rapidly (Rosenberg 1966). Only at the more rapid rate of change could recurring shadow responses be elicited and swimming frequency above maintenance swimming be reached and only then could a net upward movement be detected. This is in fact what is observed since *Polyorchis* does not show appreciable changes in its position in the water column until after sunset (Mills 1983; Chapter 2; Arkett 1984), although there is probably some time lag in their movements. This may be due to the poor temporal resolution of the field sampling. *Daphnia* shows a threshold response to the rate of light intensity decrease since the swimming reaction is not initiated until the rate of light intensity decrease reaches 0.0017 per s even though the rate of light intensity change at dusk ranged from 0.0013 to 0.0024/s (Ringelberg 1964). Stearns and Forward (1984) also found that the copepod *Acartia tonsa* did not show appreciable numbers moving upward into the water column until about 30-40 minutes after sunset. Munz and McFarland (1973) found that the "quiet period" in coral reef fishes also occurs only during the most rapid changes in light intensity, after sunset and before sunrise. Alldredge and King (1980) also showed that a wide variety of demersal plankters emerge from the benthos after dusk. These studies suggest that many animals are cueing on these slow, continuous shadows and it is possible that their threshold rate of light intensity change is species specific. However, the scarcity of information on this rate precludes any such conclusions at this time.

In addition to decreases in light intensity there is a distinct spectral shift in the light penetrating the water during sunrise and sunset, which may influence the shadow response. During sunset, the underwater spectrum tends to shift toward shorter wavelengths (450-500 nm, blue) (Munz and McFarland 1973). The peak responses of the shadow response (of smaller medusae at least) near 450 nm (Fig. 9) suggests that they are cueing on the quality of light that is most prevalent at sunset. However, the large amount of detrital matter or "yellow substance" in coastal waters (Jerlov 1966) and especially in Bamfield Inlet tends to absorb blue light and often tends to shift the maximum transmission to green (500-550 nm) which is close to the maximal shadow response for larger medusae (Fig. 9). This suggests that the resultant shadow response swimming frequencies found in the continuous change in light intensity experiments (Fig. 10) are conservative because the light quality (spectrum) was constant throughout. If I had been able to mimic spectral changes, which normally occur during sunset and sunrise, in these experiments the responses to various rates of light intensity change may have been greater. The broad spectral distribution of the shadow response (Fig. 9) appears, however, to enable *Polyorchis* to respond to light intensity reductions under a wide range of spectral conditions.

The marked inhibition of swimming by crumpling during increasing light intensity may explain why *Polyorchis* shows a downward movement toward and an aggregation near the bottom just after dawn (Mills 1983; Chapter 2; Arkett 1984). At rates of increasing light intensity representative of field conditions, the predicted swimming frequency falls well below that of maintenance swimming (Fig. 10). This alone should account for a net downward movement at dawn as *Polyorchis* is usually negatively buoyant (Chapter 6) and any reduction in swimming frequency below maintenance swimming should cause sinking. Even more important than a reduction in swimming frequency is the fact that during increasing light, severe "crumpling" occurs. This behavior is common to many medusae and it often occurs in response to noxious stimuli. Because this behavior augments normal sinking rates, it has usually been considered an escape mechanism; rapidly sinking to avoid predators. However, the progressive crumpling and resultant rapid passive sinking in response to increasing light , which often lasts the duration of increasing light intensity, suggests that this behavior may be functionally important for the dawn downward movements. Passive sinking in response to increases in light intensity at dawn also appears to be a common behavior among several groups of zooplankters (e.g., cladocerans, copepods, brachyuran larvae) (Daan and Ringelberg 1969; Forward et al. 1984; Stearns and Forward 1984; Sulkin 1984). This response, in addition to functioning as a mechanism for dawn sinking, has also been suggested as a mechanism for regulating a deep daytime position. Light intensity increases, encountered by individuals during the day, act as a barrier for upward movements (Pearre 1973; Forward et al. 1984). A similar mechanism may also maintain the deep daytime position exhibited by *Polyorchis*. This is supported by the observation that the continuous swimming seen during slow decreasing light intensity can be stopped by interposed light intensity increases. Ohtsu (1983) has suggested another mechanism by which the hydromedusan *Spirocodon* may maintain its deep daytime position. He found that UV (350 nm) light hyperpolarizes the swimming motor neurons and thereby inhibits swimming. As individuals swim upward in the water column toward the surface, he suggested that the medusae encounter increasing UV light intensities, which should inhibit swimming and eventually cause sinking.

Comparatively short wavelengths (350 nm) were not tried on *Polyorchis* and Ohtsu's explanation of sinking in surface water may apply to *Polyorchis*.

The rate of light intensity increases necessary to elicit the inhibition of swimming also appears to control the timing of the dawn sinking. As the rate of percent increase in light intensity is most rapid before sunrise (McNaught and Hasler 1964), rapid sinking due to the inhibition of swimming to a level below maintenance swimming should occur just before sunrise. This is again what is observed in the field and the effect is more dramatic than the nighttime upward movement. Figure 1 (Chapter 2) shows this effect with the largest percentage of individuals very near the bottom early in the morning.

Ontogenetic changes in photosensitivity in marine invertebrates are common and these shifts may be important in shaping the distribution and feeding behavior of later stages and adults (Thorson 1964; Pearre 1973; Forward and Costlow 1974; Cronin 1982). Differences in the responses of various sizes of medusae to light stimuli found in this study suggest that *Polyorchis* also undergoes ontogenetic changes in its photic behavior. Smaller medusae swam at a greater frequency at higher light intensities, showed a slightly broader spectral sensitivity of the shadow response with its peak shifted toward shorter wavelength, and were more responsive to rapid shadows. These findings in addition to higher maximum swimming frequency during extended swimming bouts (Fig. 7), greater maintenance swimming frequency (Chapter 2: Arkett 1984), and a greater maximum velocity (Gladfelter 1972) all suggest that smaller and presumably younger medusae are more planktonic and spend more time in the surface waters. There is also an indication that smaller medusae sink slower than larger medusae (Chapter 6). This is probably due to the fact that smaller medusae have fewer and smaller protein-laden tentacles and smaller manubria, but this may be offset by the increased lipid content of the mature gonads in larger individuals. These attributes, which maintain newly budded and young stages of medusae in surface waters, may facilitate their dispersal as well as enable them to capture and eat the predominantly smaller plankters found in surface waters. Indeed, many of the very small individuals used for these experiments were collected from surface waters. As the individuals age, many of the juvenile photic traits are altered and *Polyorchis* spends more time in deeper water and assumes a demersal existence, feeding on the large demersal plankters (Arkett 1984). Synchronized upward movement at night and concomitant spawning of these older, reproductive individuals (Mills 1983) would also ensure a higher rate of fertilization.

The use of a treadmill was essential for the controlled laboratory study of the photic behavior of *Polyorchis*. The design of this device has enabled individual medusae of various sizes to perform their entire behavioral repertoire and to respond to photic stimuli without "wall effects", which often plague tank studies. Medusan swimming activity is often increased or decreased when individuals collide with the sides of the tank. The treadmill prevents tentacle or bell contact with the walls and allows continuous, uninterrupted swimming. More importantly, the constant orientation of the photoreceptors with respect to light stimuli allows one to precisely control light conditions. One drawback of the treadmill is that swimming speed and turning behavior can not be determined, but this information can be gained from accompanying tank studies. Treadmills of various designs may also be useful for studying other behavior, such as feeding..

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IV. ELECTROPHYSIOLOGY OF THE SHADOW RESPONSE I.

The Shadow Reflex of a Hydromedusan:

I. Sequence of Events and Reflex Components

Introduction

One of the simplest forms of behavior as defined in Chapter 1 is in the form of a simple reflex. I will define the features of a reflex to include: 1) a predictable sequence of events; 2) morphologically and physiologically identifiable reflex arc components (i.e., receptors, afferent neurons, centralized integrator, efferent neurons, effector organs); 3) a graded response with respect to the intensity of a *specific* stimulus. These criteria are readily met in numerous examples of reflexes in bilaterally symmetrical systems of vertebrates and many invertebrates. However, the nervous organization of the radially symmetrical cnidarians is very different and whether or not the simplest behaviors in this group are mediated by demonstrable reflexes remains equivocal.

One example of a simple behavior exhibited by some members of the cnidarians, the hydromedusae, that may be a demonstrable reflex, is the shadow response. This behavior consists of a few rapid swimming contractions and simultaneous contractions of tentacles in response to a rapid reduction in light intensity (Murbach 1909; Hisada 1956; Singla 1974; Mackie 1975). The scarcity of examples where discrete neuronal units have been physiologically identified and the general conception that this group lacks any centralized region of the nervous system has, until recently, precluded the idea that a simple behavior, like the shadow response, could be a reflex as previously defined. However, within the last few years, intracellular recording techniques have been used successfully on medusae and the cellular mechanisms of the components involved in simple behaviors are being examined.

Several functional units involved in the shadow response have been identified in the hydromedusan *Polyorchis penicillatus*. Intracellular recording from large neurons within the inner nerve-ring (INR) by Anderson and Mackie (1977), Spencer and Satterlie (1980), Spencer (1981) identified these neurons as a part of an anastomotic network of electrically-coupled swimming motor neurons (SMNs). Following a shadow, these SMNs depolarize under a barrage of excitatory post-synaptic potentials (EPSPs) and fire a burst of 1-4 action potentials. Each spike in the burst produces a contraction in swimming muscles and hence one swimming stroke. This response is obliterated if the ocelli are removed suggesting that photic information originates in the ocelli (Anderson and Mackie 1977). The cilium-based receptor cells of the ocelli (Eakin and Westfall 1962) form chemical synapses onto purported secondary neurons which enter the outer nerve-ring (ONR) (Singla and Weber 1982a). Spencer and Arkett (1984) identified two networks ("B" and "O" systems) of electrically-coupled neurons in the outer nerve-ring (ONR). Both of these systems showed a marked response to shadows, which suggested their involvement in the shadow response. This finding was significant because for the first time the cellular properties of the neurons within the ring-shaped central nervous system were described and their role as the integrating center of sensory information was supported. Heretofore, the ONR had been suspected as the CNS of hydromedusae (Romanes 1876; Hertwig and Hertwig 1878), but little was known of its organization because intracellular recordings had never been made from the systems within it. Thus, identification of these and other systems now generates several questions about the simplest form of behavior in this radially symmetrical animal. What is the sequence of events in the shadow response and are they predictable? Are the systems which are involved in the shadow response part of an identifiable reflex arc?

I describe here the responses of several electrically-coupled neuronal networks and effector units involved in the shadow response of the hydromedusan *Polyorchis penicillatus*. Intracellular recordings from the various components show the predictability of the timing and sequence of events involved in the shadow response. Removal of the ocelli shows that these structures and their connections to the ONR are important for the production and execution of the shadow response. Isolation of each network from synaptic inputs by $MgCl_2$ anesthesia shows the endogenous photosensitivity of only the "O" system. The shadow response and the organization of the networks involved illustrate a fundamental reflex arc, starting in the ocelli and terminating with the swimming and tentacle muscles. In Chapter 5, I demonstrate the graded nature of the shadow response, consider how photic information might be integrated in a radially symmetrical animal, and discuss the functional significance of the shadow response.

Methods and Materials

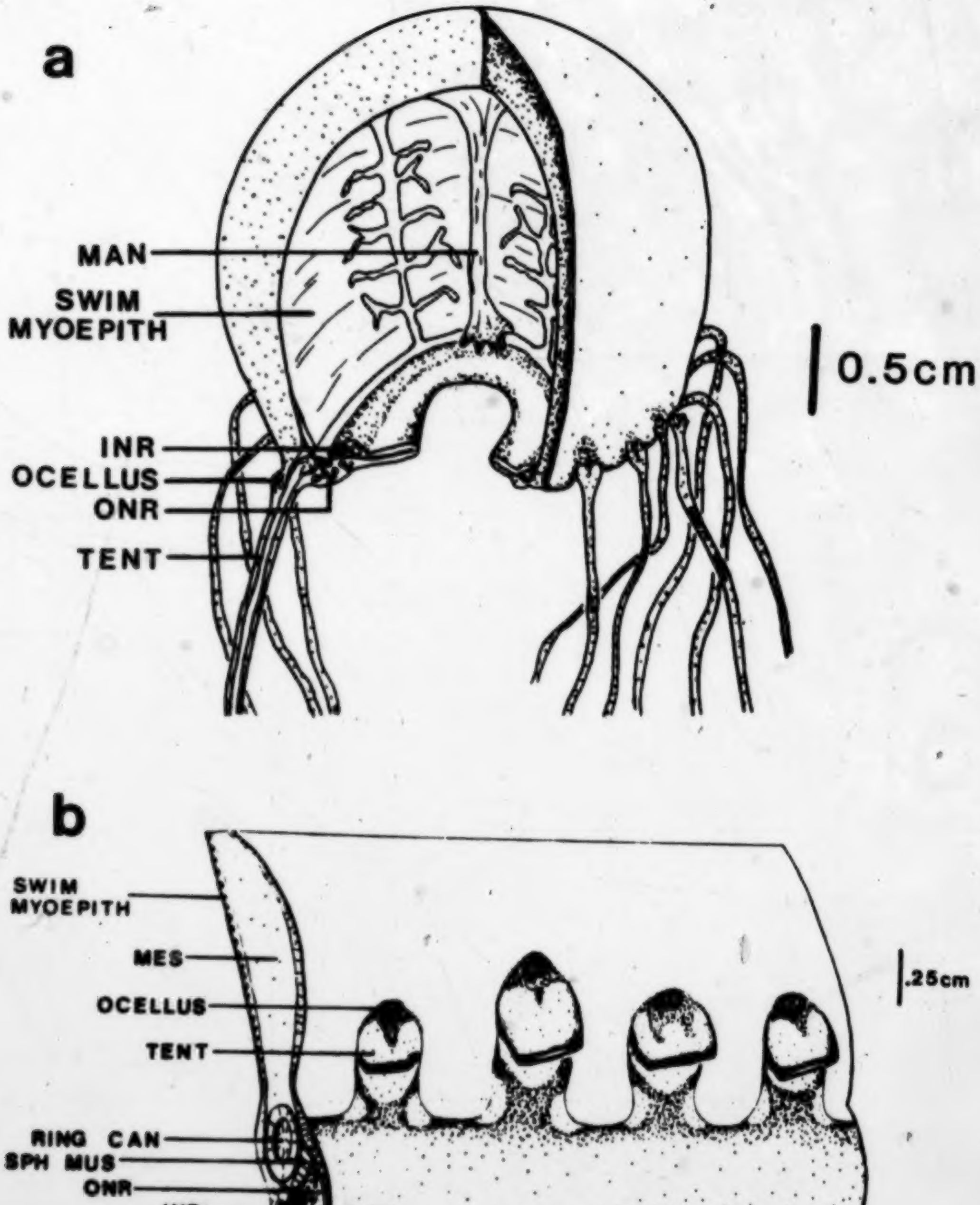
Individual hydromedusae of *Polyorchis penicillatus* were collected by divers from Bamfield Inlet, Bamfield, B.C.. Medusae were kept in running seawater (10-12 °C) and were used for experiments within 3-5 days. Medium-sized individuals with bell heights of 1-3 cm were routinely used. All recordings were made in natural seawater (15-18 °C) unless otherwise stated.

The outer nerve-ring preparation (Fig. 11) and conventional intra- and extracellular recording techniques have been described previously (Spencer 1978, Spencer and Arkett 1984). For "intact" experiments, only the distal portion of each tentacle was removed to ensure that the ocelli and their connections to the outer nerve-ring remained undisturbed. In some experiments requiring ablation of the ocelli, a small portion of the exumbrellar mesoglea, including all of the ocelli and all but the most proximal portion of the tentacles, was cut away. The nerve-ring remained undamaged. A 1:1 mixture of isotonic (0.33 M) $MgCl_2$ and seawater was used as an anesthetic for experiments requiring blockage of chemical synapses. The preparation was kept in this solution at least one half hour before beginning any experiments.

Photic stimulation consisted of rapid changes in illumination. The preparation was illuminated from below by a fiber optic system connected to a quartz halogen source. A mechanical camera shutter, placed between the source and the fiber optic, was held open by a cable release during light ON recordings. At light OFF, the shutter was released, closing within 2 ms, to present a "shadow". All of the light changes were approximately 100% with light intensity at the level of the preparation during light ON at approximately 200-300 microeinsteins/ m^2 s. This was reduced to less than 0.1 microeinsteins/ m^2 s at light OFF. A photocell located beneath the preparation monitored changes in illumination. The unpredictability of the neuronal penetrations and difficulty in maintaining penetrations precluded any attempts to control adaptation times before shadow stimulation.

The distribution of the three neuronal systems ("O", "B", SMNs) have been identified previously by Lucifer Yellow iontophoresis (Spencer and Satterlie 1980; Spencer and Arkett 1984). Figure 12 shows the distribution of these systems in the inner and

Figure 11. a) Schematic illustration of *Polyorchis* with a cut away portion of a whole individual showing the radial array of tentacles and ocelli and the orientation of the nerve-rings on either side of the velar sheet. The cut-away portion has been enlarged. b) A portion of the bell as it appears in the "intact" recordings with the ocelli and exumbrellar surface of the bell upward. Tentacles have been removed just distal to the ocellar cup. INR, inner nerve-ring; ONR, outer nerve-ring; SWIM MYOEPITH, swimming myoepithelium; TENT, tentacle; VEL, velum; SPH MUS, sphincter muscle; RING CAN, ring canal; MES, mesoglea; ONR EPITH, outer nerve-ring epithelium; OCELLUS; VEL MUS, velar muscle; MAN, manubrium.


a
MAN
SWIM MYOEPITH
0.5cm
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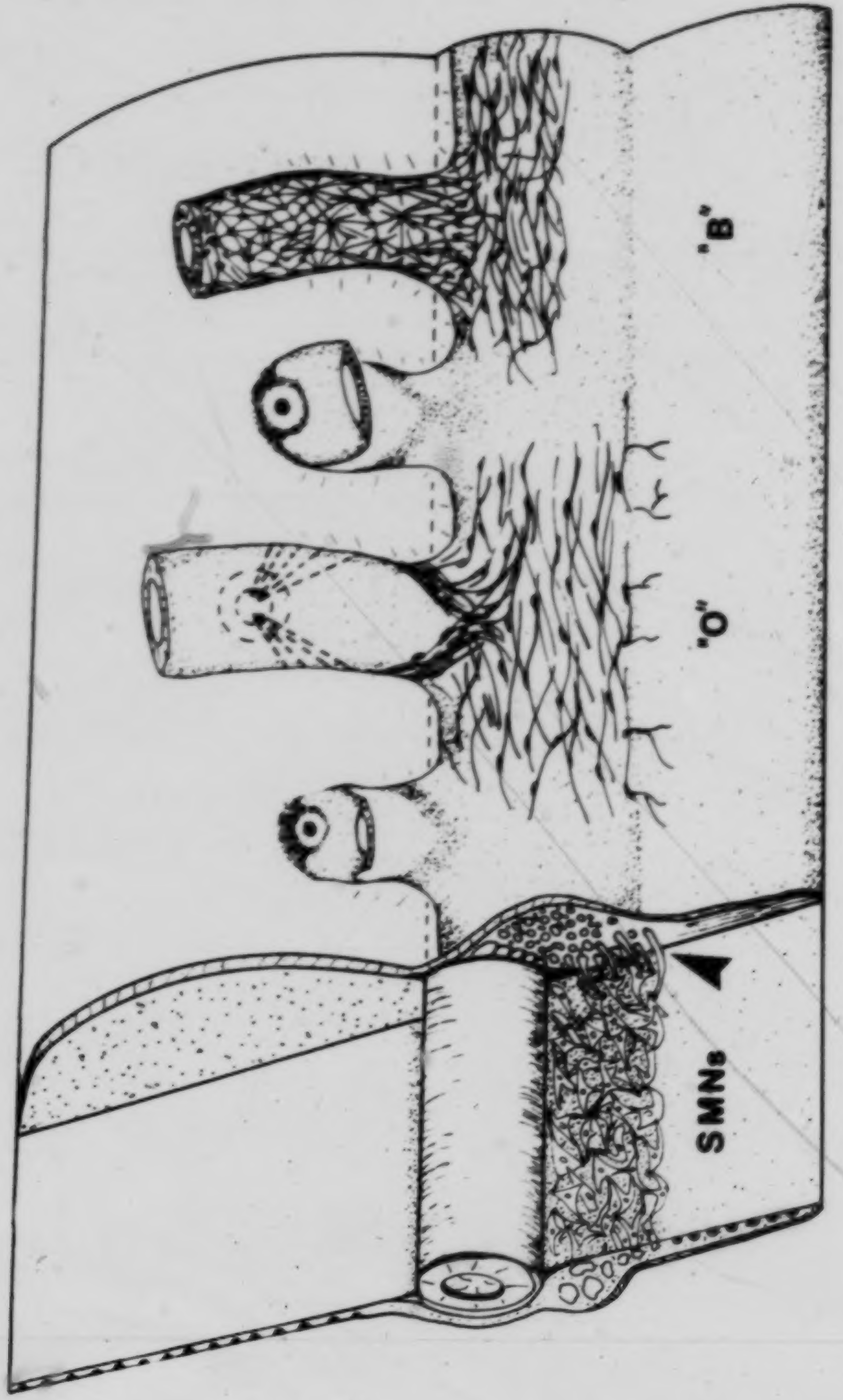
VEL

outer nerve-rings and in the tentacles. "B" system neurons were identified by their resting potential (about -40 mV), their spiking activity correlated with tentacle contractions, and their bursting activity in response to a shadow. The "B" system appears to be located superficially since it was usually encountered just beneath the ONR epithelium. "O" system neurons were identified by their characteristic non-spiking, regular oscillations of the membrane potential and by their rapid hyperpolarization (without spiking) in response to rapid shadows. The membrane potential of the "O" system oscillates about 20 mV above and below a resting potential of between -40 and -75 mV (Spencer and Arkett 1984). The "O" system appears to be much deeper in the ONR as it was usually encountered after passing through the "B" system. SMNs were penetrated from the ONR side by advancing the electrode through the ONR, past the mesoglea that separates the inner and outer nerve-ring, and into the INR. SMNs were identified by their resting potential (about -60 mV), their spiking activity in response to shadows, and by their 1:1 correlation with swimming muscle contractions.

Figure 12. Schematic diagram of the distribution and morphology of the neuronal systems involved in the shadow reflex. The "B" system extends over part of the ONR and sends a plexus of neurons up each tentacle, extending to the tip. The "O" system extends over a wider portion of the ONR and sends two bundles up and around each tentacle. These bundles terminate at the ocellus, which in this figure is behind the folded-back tentacle. A portion of the ONR has been cut away to expose the SMNs located in the INR. The arrow indicates how ONR neurons cross the mesoglea and synapse onto the SMNs.


"B"
"O"
SMNs

Results

Response of SMNs to Light Intensity Changes

Intracellular recordings from the swimming motor neurons (SMNs) in the "intact" preparation show fairly regular, spontaneous spiking activity in lighted conditions. At light OFF, the SMNs begin to depolarize after a mean delay of 154.6 ± 3.8 ms (± 1SE; n=29 from 5 individuals) and spike after approximately 200 ms. During this depolarization a barrage of excitatory post-synaptic potentials (EPSPs) can be seen to lead to a burst of one to three or four action potentials (Fig. 13a and Anderson and Mackie 1977). If the SMNs produce a burst of spikes, the shortest interspike interval between the first 2 APs is about 400ms; thereafter, the interval increases (Figs. 13a and 14). During this burst of spikes there is a slow, underlying depolarization which usually lasts the duration of the spiking activity. This is followed by a slow hyperpolarization, during which time the SMNs are silent except for small unitary EPSPs, primarily from the "B" system (Spencer and Arkett 1984). These EPSPs do not usually result in action potentials in the SMNs during the hyperpolarization. SMNs adapt to the dark conditions and usually begin to fire spontaneously after 15-20 seconds. At light ON, the SMNs initially hyperpolarize and then depolarize and resume spiking (Fig. 13a and Anderson and Mackie 1977). The latency for the ON response is often variable and sometimes requires several seconds before firing begins.

Removal of the ocelli and tentacles does not disrupt the firing frequency of the SMNs in lighted conditions, however, at light OFF, the response is very different from the "intact" preparation. At light OFF, there is no depolarization and associated burst of action potentials; instead the SMNs hyperpolarize after a longer and more variable latency (approximately 1s) (Fig. 13b). The membrane potential of the SMNs remains hyperpolarized during darkened periods with EPSPs still visible. The presence of these EPSPs indicate that synaptic connections between the SMNs and other systems, probably within the ONR, are still intact. These connections cross the layer of mesogloea separating the two nerve-rings (Spencer 1979). At light ON, the SMNs depolarize after several seconds and return to their previous spiking frequency.

Figure 13. Intracellular recordings from the swimming motor neurons (SMNs) in the inner nerve-ring of *Polyorchis* with a light trace (lower trace) showing changes in light intensity. Downward deflection (duration 2 ms) indicates a 100% shadow. Light traces have been redrawn from original traces. a) "Intact" preparation showing normal response to rapid decrease in light intensity. Note the burst of action potentials and subsequent hyperpolarization. Arrow indicates EPSPs which most probably are from the "B" system. b) Response of SMNs to a rapid shadow after the ocelli and a portion of the tentacles have been removed. Note the absence of the burst of action potentials, but presence of synaptic activity. c) SMN recording in $MgCl_2$ anesthesia showing that there is no consistent, immediate response to light intensity changes. Notice the smooth membrane oscillations and the lack of PSP activity.

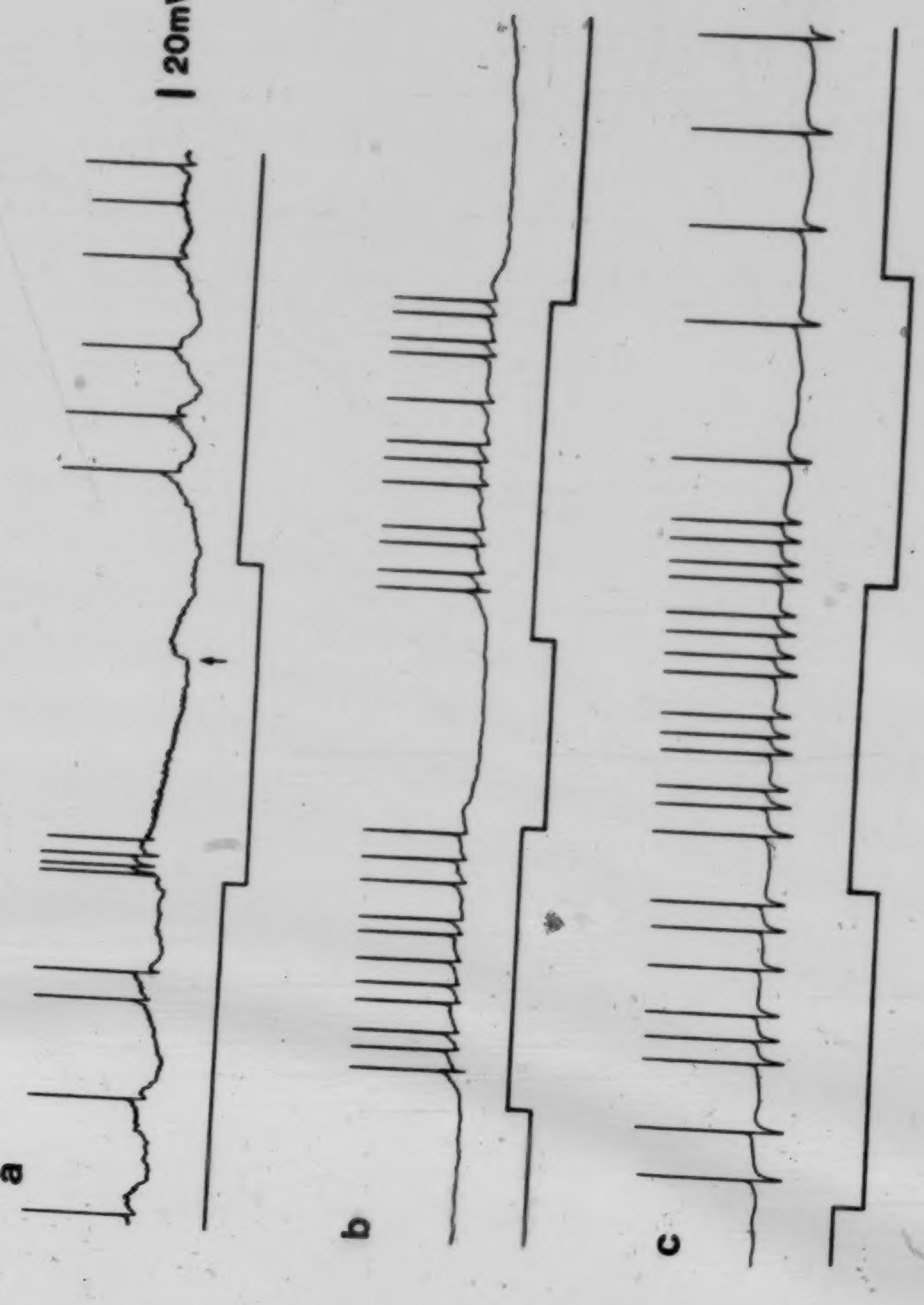
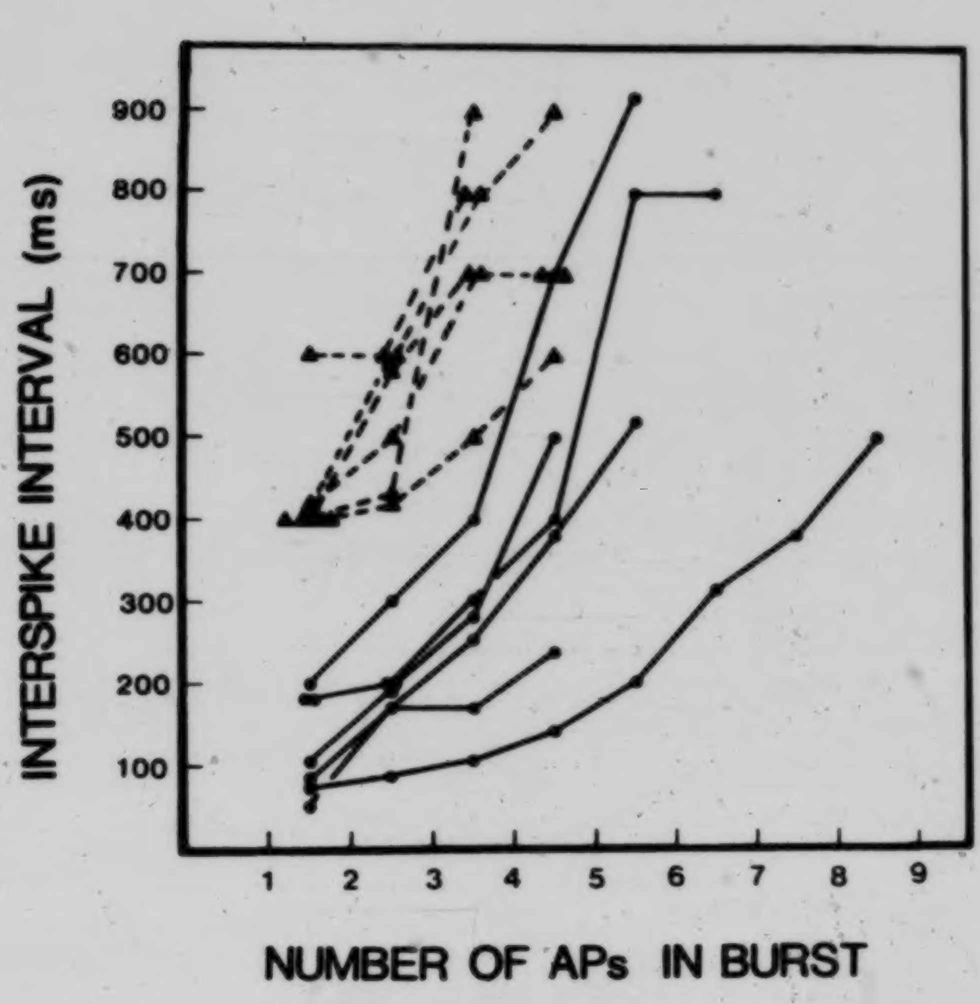

a
20mV
b
20mV
c
20mV
2s

Figure 14. Plot of the interspike interval of normal OFF response bursts in SMNs (▲) and "B" system (●). Times were taken from intracellular recordings from 7 individuals for "B" and 6 individuals for SMNs. Note that there are usually fewer total number of APs in the SMN OFF response than in the "B" system response and also the interspike interval is generally longer for the SMNs than for the "B" system for the same number of APs in a burst. SMNs appear to show a minimum interspike interval of 400 ms between the first and second spike in a typical burst. The minimum interval for the first two spikes in a "B" system burst appears much shorter, usually about 100 ms or less.


INTERSPIKE INTERVAL (ms)
900
800
700
600
500
400
300
200
100
1
2
3
4
5
6
7
8
9
NUMBER OF APs IN BURST

The SMNs failed to show any detectable response to changes in light intensity after isolating them from all synaptic input by using $MgCl_2$ anesthesia (Fig. 13c & 20e). The firing frequency of the SMNs in $MgCl_2$ anesthesia was either fairly regular or silent during lighted periods. No PSPs were visible. At light OFF, there was no change either in firing frequency or membrane potential. The anesthetic effect of $MgCl_2$ was reversible as "intact" individuals showing no photic response under anesthesia, showed a normal bursting response to light OFF.

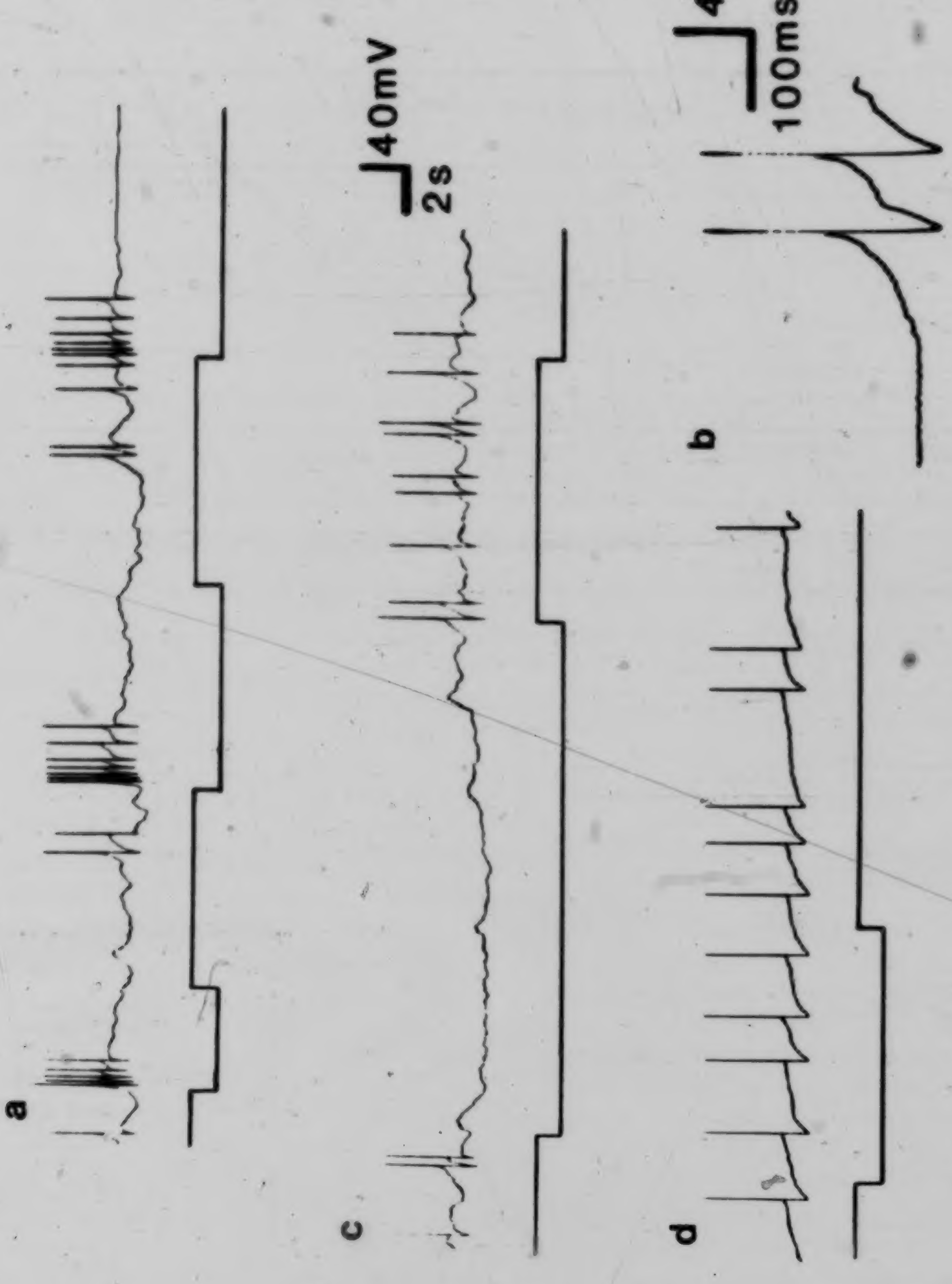
Responses of "B" system to Light Intensity Changes

Recordings from the "B" system in the ONR of intact preparations during light ON show a fairly regular spontaneous firing frequency. At light OFF, the "B" system also begins to depolarize after a mean delay of 167.6 ± 3.6 ms (± 1SE; n=7 from 5 individuals) with the first action potential occurring after the first SMN spike at around 220-300 ms (Fig. 15a and b). The OFF response consists of 4-8 spikes with the interspike interval rapidly increasing. The interspike interval for the first two action potentials is shorter than that observed in the SMNs (Fig. 14) starting around 100 ms and extending to 400-500ms after 4-5 spikes. The "B" system burst is often completed before the 2nd or 3rd swimming contraction. During this burst of action potentials there is a slow 10-20 mV underlying depolarization which lasts the duration of the burst. The "B" system hyperpolarizes after the burst, but usually adapts to the dark and gradually depolarizes. At light ON, the system depolarizes and begins to spike at the previous frequency. Like the SMNs, the latency for the ON response is variable and long (several seconds) relative to the OFF response.

Ablation of the ocelli and tentacles has the same effects on the "B" system as this operation did on the SMNs. Under normal lighting conditions, the "B" system spikes at a similar frequency as the intact preparation. At light OFF, there is a 10-20 mV hyperpolarization without the high frequency burst of action potentials (Fig. 15c). This hyperpolarization continues in the dark; at light ON, the "B" system depolarizes and resumes firing at its previous frequency.

Recordings from the "B" system in $MgCl_2$ anesthesia, like the SMNs, show no detectable response to changes in light (Fig. 15d). All PSP activity is absent, but the

Figure 15. Intracellular recordings from the "B" system network in the ONR of *Polyorchis* with the light trace below. a) Response of the "B" system to a rapid shadow in the "intact" preparation. Notice the high frequency burst of action potentials on the underlying slow depolarization. b) Oscilloscope trace showing delay of the "B" system after light OFF. Sweep was triggered by the light monitor. c) Shadow response of the "B" system after the ocelli and tentacles have been removed. Like the SMNs there is a marked absence of the bursting of APs at light OFF. Synaptic activity is still apparent, probably coming from other systems in the ONR. d) "B" system recording with all synaptic activity blocked by $MgCl_2$ anesthesia. There is no detectable response to shadows.


a
b
c
d
40mV
2s
40mV
100ms

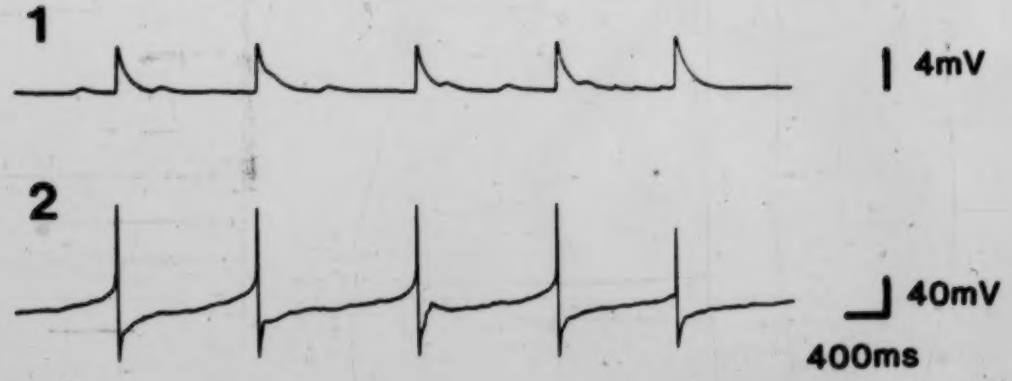
system appears to have some endogenous rhythmicity as it fires at a fairly regular frequency.

Recordings from Myoepithelial Effectors of the Tentacles and ONR

Intracellular recordings from the ectodermal myoepithelium of the tentacles show unitary EPSPs that can be correlated 1:1 with "B" system action potentials (Fig. 16). Simultaneous recordings from widely separated tentacles show the synchronizing effects of the electrically-coupled (Spencer and Arkett 1984) "B" system (Fig. 17). Each EPSP is associated with a local tentacle contraction. This connection is chemical in nature as bathing the preparation in $MgCl_2$ anesthesia reversibly abolishes the EPSPs. "B" system spikes also produce unitary EPSPs in the ectodermal epithelium overlying the ONR (Spencer and Arkett 1984) and show the same latency as to the tentacle myoepithelium (Fig. 18). EPSPs seen in the ONR epithelium are, however, slightly attenuated relative to those seen in the tentacle myoepithelium. This may be due to the relatively deeper location of the "B" system in the ONR as compared to the shallow plexus of the "B" system in the tentacle. At light OFF, EPSPs from both the tentacle myoepithelium and the ONR epithelium summate, during which time, the tentacles can be seen to rapidly shorten in consecutive contractions.

Recordings from the ONR epithelium show additional activity which appears to consist of overshooting action potentials. These "spikes" although of large amplitude relative to the "B" system-generated EPSPs, do not appear to be a result of "B" system spikes because they occur out of phase with them. This activity is most apparent during the shadow response (Fig. 19), but can occur spontaneously in singles or in multiples. These "spikes" are most obvious in the ONR epithelium, but they are also present in the tentacle myoepithelium, albeit severely attenuated (Fig. 19). Their presence in the ONR epithelium suggests that these action potentials originate in the ONR epithelium or from specialized muscle groups near the ONR, such as velar radial muscle and sphincter muscle. Spencer (1978, 1981) and King (1979) have recorded similar activity from radial muscle, radial canal, and endoderm, all of which may be involved in the severe contractions of the tentacles and involution of the bell margin during "crumpling". The attenuation of the amplitude of these ONR epithelium "spikes" suggests that they may not be overshooting

Figure 16. Dual intracellular recording from the myoepithelium of the tentacle (1) (resting potential about -40 mV) and from the "B" system in the ONR at the base of the same tentacle (2). The most stable recordings for the tentacle myoepithelium were very shallow within the center of the ocellar cup, although identical recordings can be obtained from more distal portions of the tentacle. Delay from peak of "B" action potential to start of depolarization in tentacle epithelium is constant at 8-10 ms.


1
4mV
2
40mV
400ms

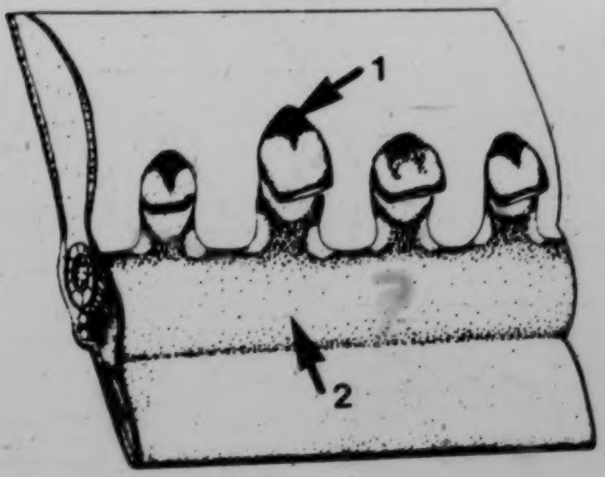

1
2

Figure 17. a) Widely spaced (>1.0 cm) simultaneous intracellular recordings from the myoepithelium of two tentacles recorded at the ocellus. During light ON, EPSPs are synchronized by the "B" system spikes. At light OFF (large arrow), both traces show summing EPSPs due to a rapid burst of the "B" system. Small arrows in bottom trace indicate the presence of some local input not seen in the upper trace. b) Recording from the same position as in (a), but after a break (dashed line in inset) in the ONR was made. Although the response to shadows is intact, there is a noticeable asynchronization in the "B" system-generated EPSPs due to the ONR disruption. Notice that the delay from light OFF (large arrows) to the first depolarization of EPSPs is also asynchronized at the two different recording sites.

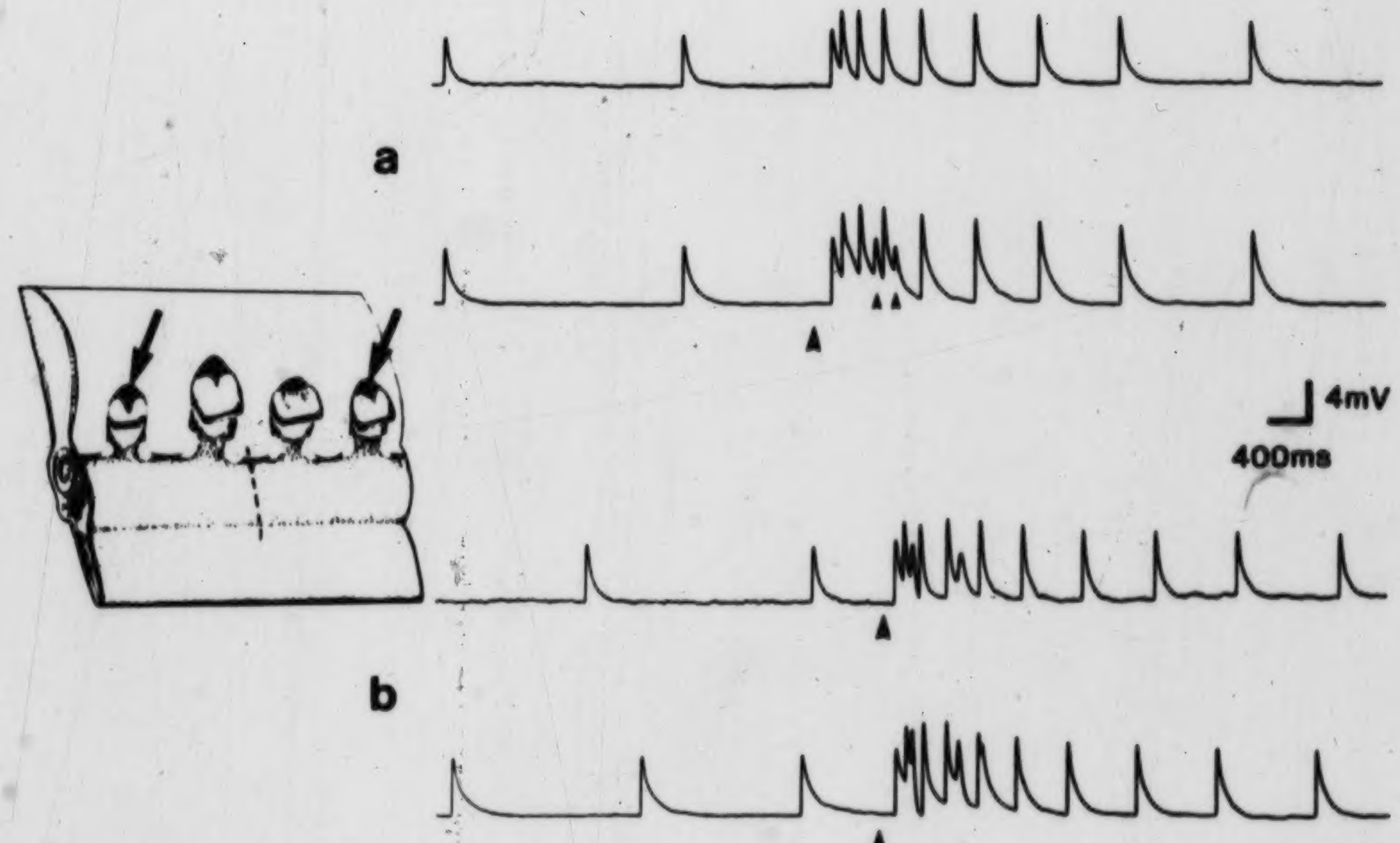
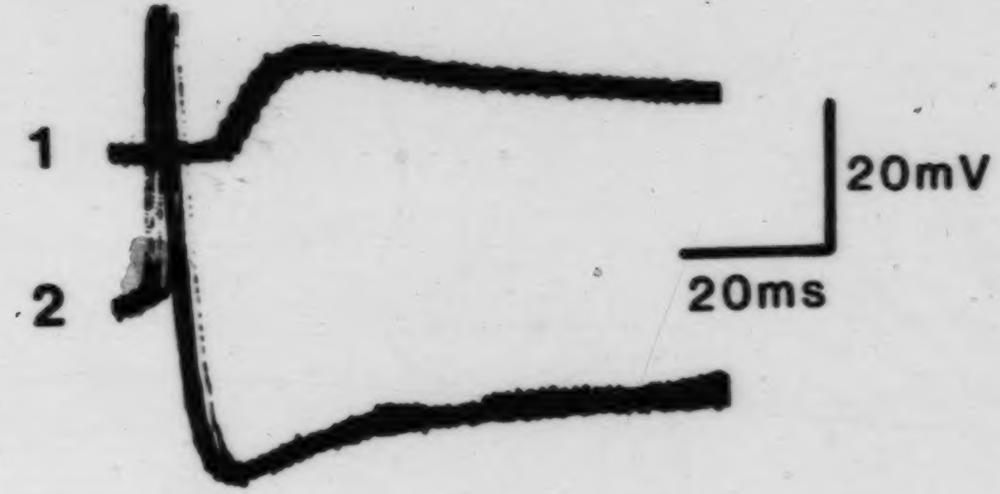

a
b
4mV
400ms

Figure 18. Dual intracellular recording from ONR epithelium (1) (resting potential about -40 mV) and "B" system in the ONR (2). Recordings were made within one tentacle of each other. Six oscilloscope sweeps show the short and constant delay of the EPSPs in the epithelium which is similar to the delay between the "B" system and tentacle myoepithelium.


1
2
20mV
20ms

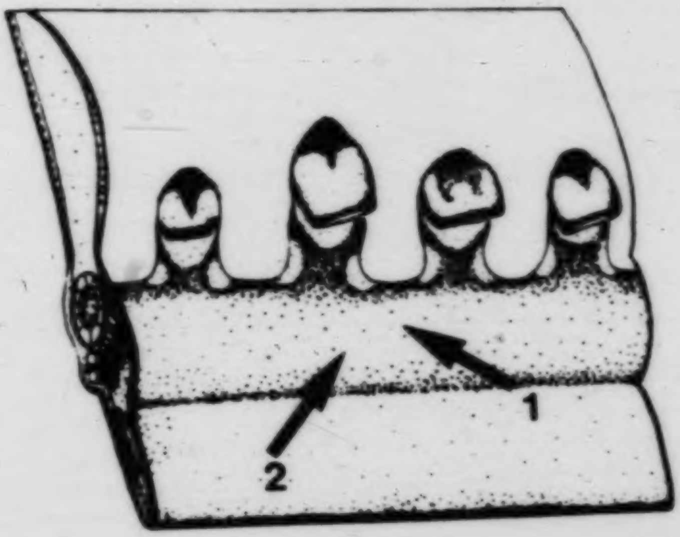
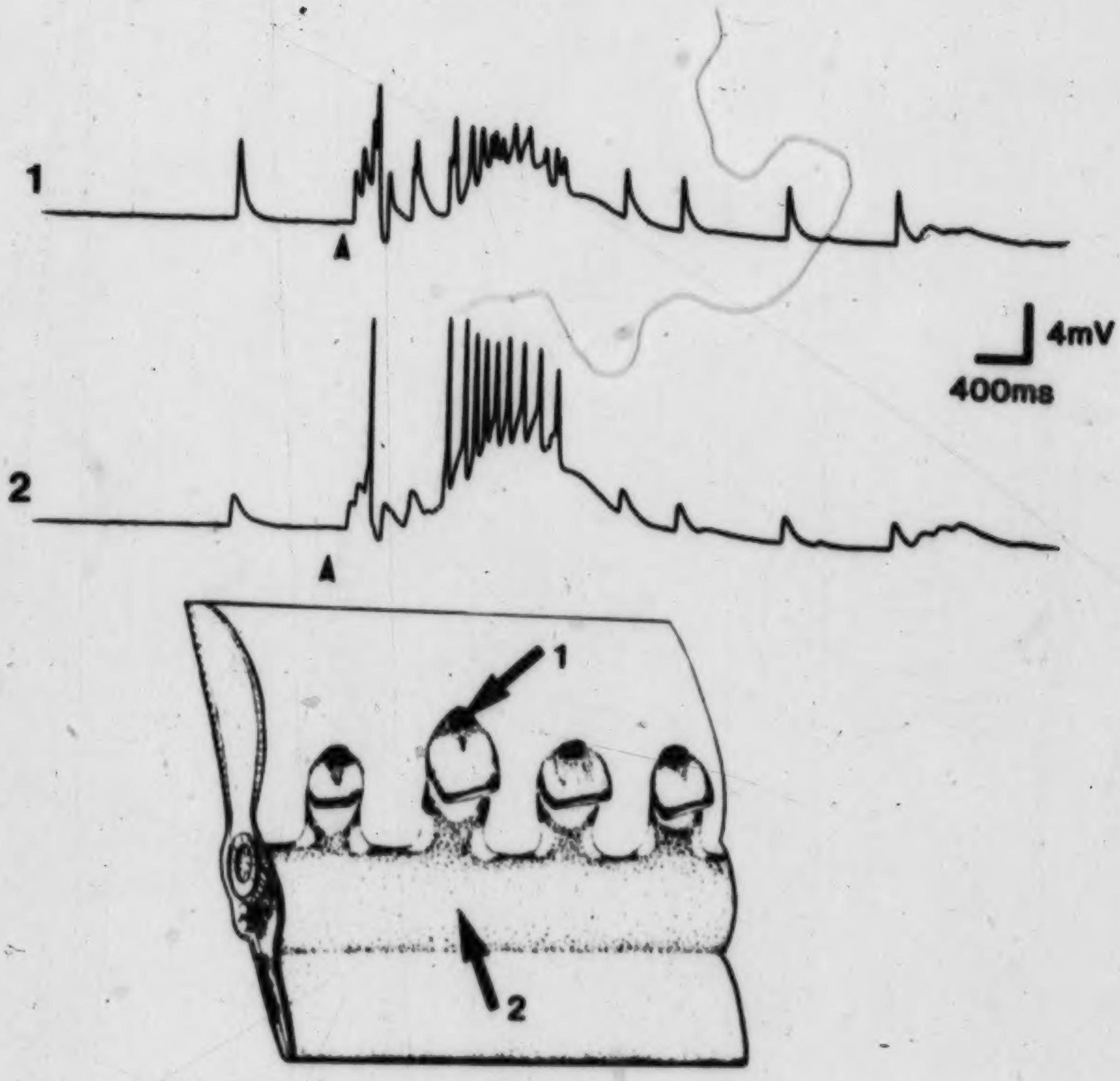

1
2

Figure 19. Dual intracellular recording from the tentacle myoepithelium (1) and from the ONR epithelium directly below that tentacle (2). Notice that EPSPs are synchronized due to common input from the "B" system, but that the ONR epithelium EPSPs are somewhat attentuated. At light OFF (arrows), both systems depolarize with the EPSPs recorded at the ocellus (1) slightly leading the EPSPs in the ONR. This suggests that the OFF response is initiated at the ocellus. EPSPs sum in both systems with "spikes" most obvious in the ONR epithelium. These "spikes" are severely attenuated in the tentacle myoepithelium, but produce additional EPSPs, which are unattributable to the "B" system.


1
2
4mV
400ms
1
2

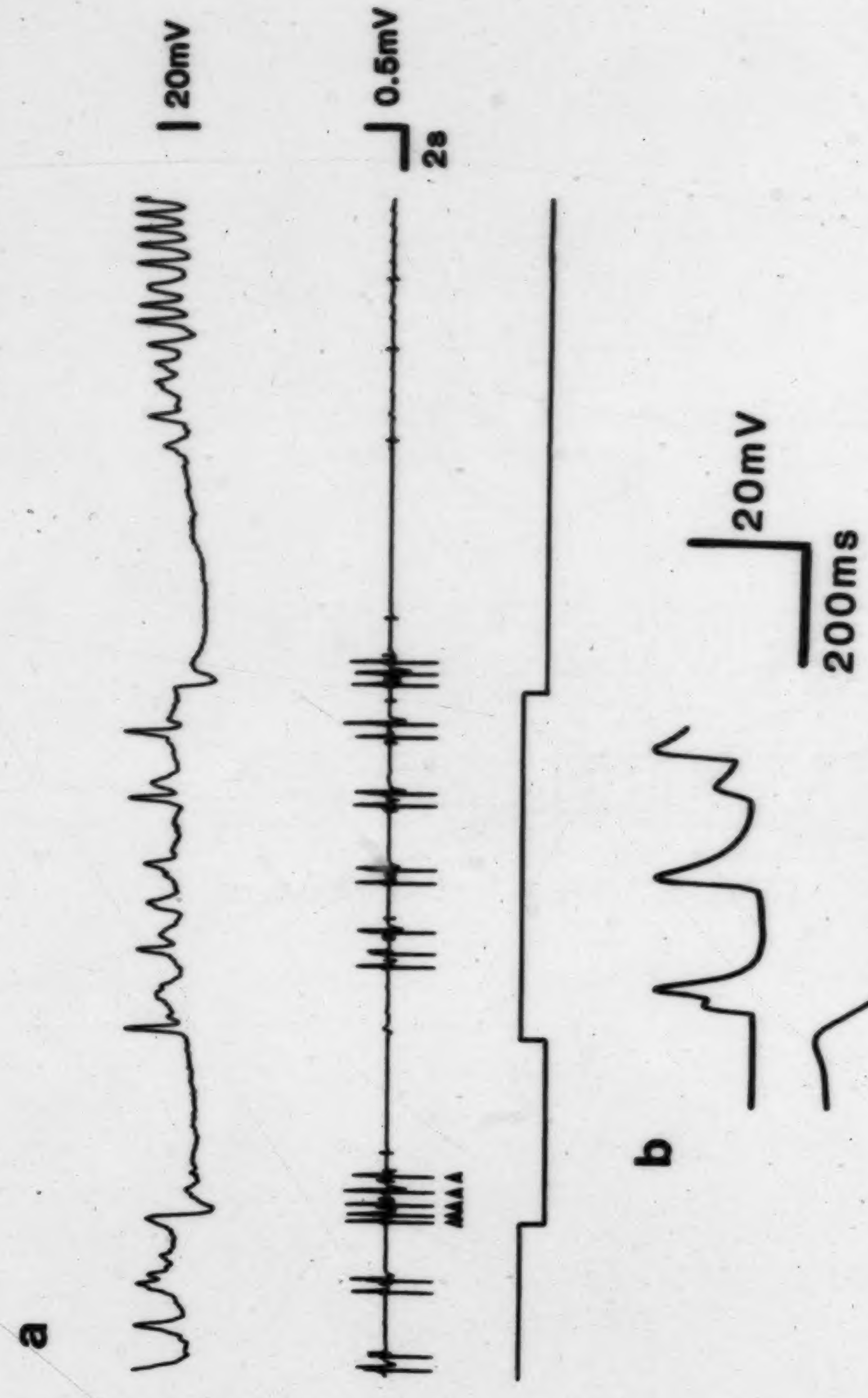
actions potentials, and their attenuation is due to decremental conduction through the electrically-coupled epithelium from the ONR to the tentacles. These additional depolarizations by the myoepithelium-generated "spikes" may augment tentacle contractions by adding onto the already summing EPSPs produced by the "B" system. This additive effect may be most important during "crumpling" when tentacle contraction is most severe. These same ONR epithelium "spikes" also produce long duration inhibitory post-synaptic potentials in the SMNs (Spencer 1981, Spencer and Arkett 1984). This also suggests that these "spikes" are involved in the crumpling behavior as the SMNs are inhibited during crumpling. The marked presence of the "spikes" in the proximal portion of the tentacles may also enable *Polyorchis* to maintain the "sink-fishing" posture (Fig. 5 Chapter 2). The proximal portion of each tentacle is held outward while more distal portions of the tentacle is relaxed and drops downward.

Responses of the "O" System to Light Intensity Changes

With the ocelli and tentacles intact, the "O" system typically shows very regular, non-spiking membrane potential oscillations at a frequency of approximately 1 Hz. These oscillations are disrupted at light OFF (Fig. 20a and Spencer and Arkett 1984) with a 10-20 mV hyperpolarization after a mean delay of 153.2 ± 3.7 ms (±1SE; n=18 from 6 individuals) (Fig. 20b). No action potentials have ever been observed in this system and no bursting activity is seen at light OFF. At light ON, the "O" system depolarizes and the oscillations resume their previous frequency. The delay of the ON response is fairly constant and is similar to that of the OFF response. This is in contrast to the SMNs and "B" system whose ON latencies are longer and more variable than their OFF response latencies. Initial oscillation frequencies after the light ON are slightly higher than pre-shadow frequencies, but the oscillations return to their regular frequency within several seconds. If the preparation is left in the dark, the "O" system appears to adapt as oscillations spontaneously reappear after 5-15 seconds (Fig. 20a). These oscillations are initially of low amplitude and low frequency, but gradually return to their previous values.

Neuro-neuronal chemical connections, which can be reversibly blocked by $MgCl_2$ anesthesia, between the "O" system and the "B" system and SMNs are extensive. Spencer and Arkett (1984) showed that both the "O" system and SMNs received simultaneous

Figure 20. a) Intracellular recording from the "O" system in the ONR (top) and extracellular suction electrode recording from the ONR (bottom) showing the response of an "intact" animal to rapid light intensity changes. At light OFF, five swimming contractions (arrows) can be seen as a response in the SMNs as the "O" system rapidly hyperpolarizes. At light ON, the "O" system rapidly depolarizes and shows the exaggerated initial oscillations. The second light OFF shows an apparent adaptation of the "O" system to the dark as oscillations spontaneously return to their initial resting potential and frequency. b) Inset shows triggered "O" system hyperpolarization at light OFF. Top trace shows "B" system-generated EPSPs recorded from the tentacle myoepithelium. Sweep was triggered by the light monitor. Arrows indicate synaptic input onto the "O" system from "B" system spikes.


a
20mV
0.5mV
2s
b
20mV
200ms

discrete EPSPs and suggested that they had a common origin. These EPSPs in the "O" system are seen most often during the strong hyperpolarization of the OFF response (Fig. 20b). It is clear that it is the "B" system which has common inputs to both the "O" and SMNs. The SMNs also feed back onto the "O" system as well because SMN spikes can alter the frequency of the "O" system by interpolating oscillations (Spencer and Arkett 1984). This type of input and its importance is more evident when action potentials from the SMNs can initiate oscillations in the "O" system when it is hyperpolarized after light OFF (Fig. 20c).

Removal of the ocelli and tentacles does not affect the normal oscillation frequency of the "O" system in light, and the OFF response is only slightly disrupted. At light OFF, the system again hyperpolarizes and the oscillations stop (Fig. 20c). The delay of the OFF response is, however, more variable and generally longer (1-1.5 s) than the intact preparation. At light ON the system depolarizes and returns to its regular frequency. Another response occasionally observed is that instead of a hyperpolarization and absence of oscillations, there is a significant decrease in the oscillation frequency at light OFF (Fig. 20d). In representative trials taken from several individuals the mean frequency (±1SE) of oscillations at light ON was 0.855 ± 0.006 Hz ($n=141$) and at light OFF was 0.758 ± 0.008 Hz ($n=153$). In both conditions the oscillations were very regular as indicated by the low standard error. One-way ANOVA (Sokal and Rohlf 1969) shows that the mean frequency of oscillations at light OFF is significantly ($p < 0.001$) lower than the oscillations at light ON.

When the "O" system is isolated by $MgCl_2$ anesthesia, it is the only one of the three systems studied that shows an immediate response to light intensity changes (Fig. 20e). During light ON, no PSP activity is visible and the oscillations, which are unique to the system, are absent or severely attenuated. Other than the attenuation of oscillations, the system behaves as the "intact" system. At light OFF, the "O" system hyperpolarizes with the delay from light OFF to the initial hyperpolarization only slightly longer (approximately 280 ms) than the normal delay. No oscillations are observed during light OFF, and at light ON, the system depolarizes after a delay similar to the OFF response. The "O" system also appears to adapt to dark conditions as it gradually depolarizes and returns to the pre-shadow membrane potential (Fig. 20e). When the "O"

Figure 20. c) Intracellular recording from the "O" system (top), SMNs (middle), and an extracellular electrode over the ONR (bottom) showing the effects of a shadow on the systems without the ocelli. Large biphasic complex pulses in the extracellular trace correspond to SMN spikes and swimming muscle contractions. Both the "O" and SMNs hyperpolarize at light OFF. 20 nA of positive current (arrow) was injected into the SMN system causing an SMN spike and a swimming contraction (seen in the third trace) which produces a single EPSP in the "O" system (top) and rapidly restarts the oscillations.

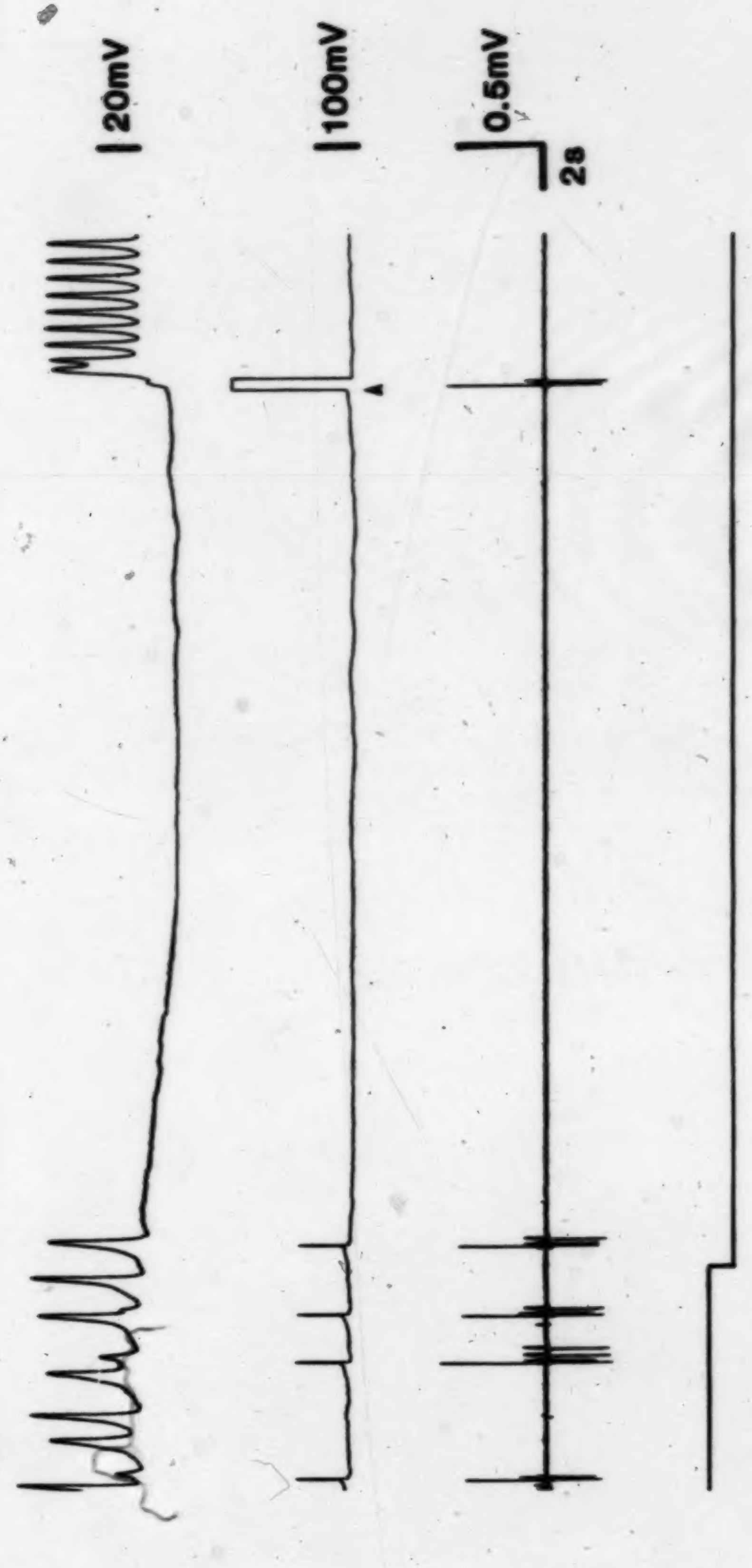

C
20mV
100mV
0.5mV
2s

Figure 20. d) Intracellular recording from the "O" system (top), extracellular recording from the ONR (middle), and light trace (bottom) from a preparation with the ocelli and tentacles removed. Notice the "O" system shows a marked reduction in oscillation frequency at light OFF with a general broading of the trough of the oscillations. "O" system oscillations can be seen in the extracellular trace and the large biphasic pulses are swimming contractions.

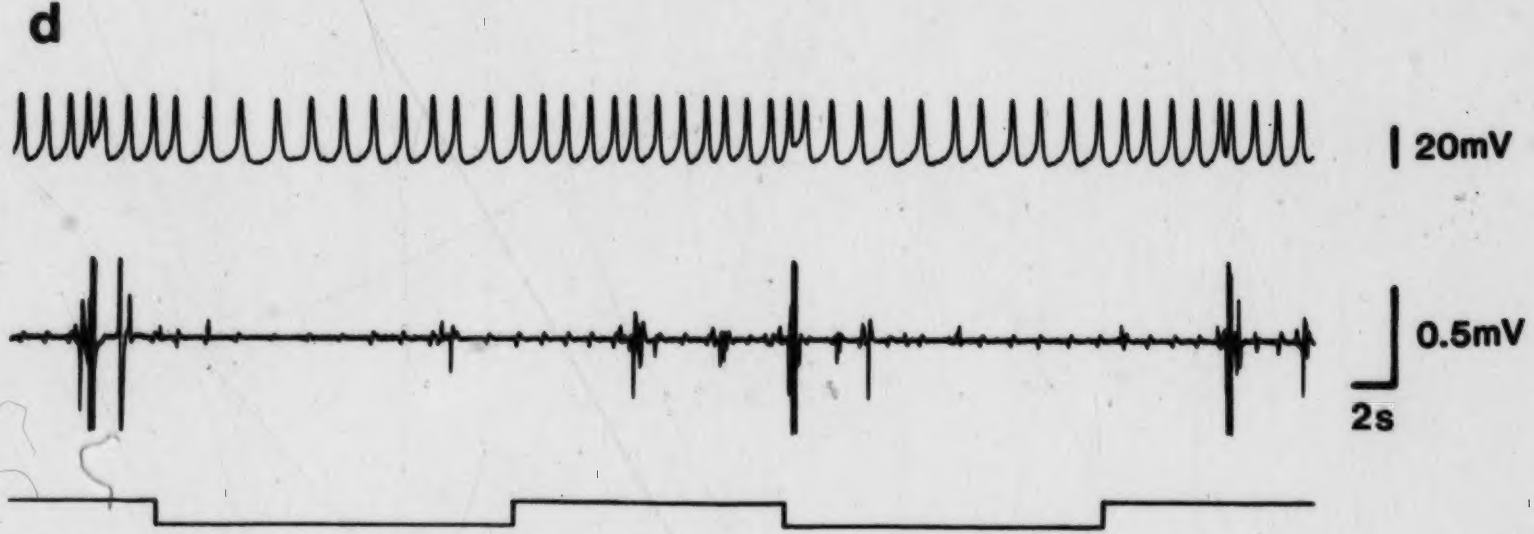
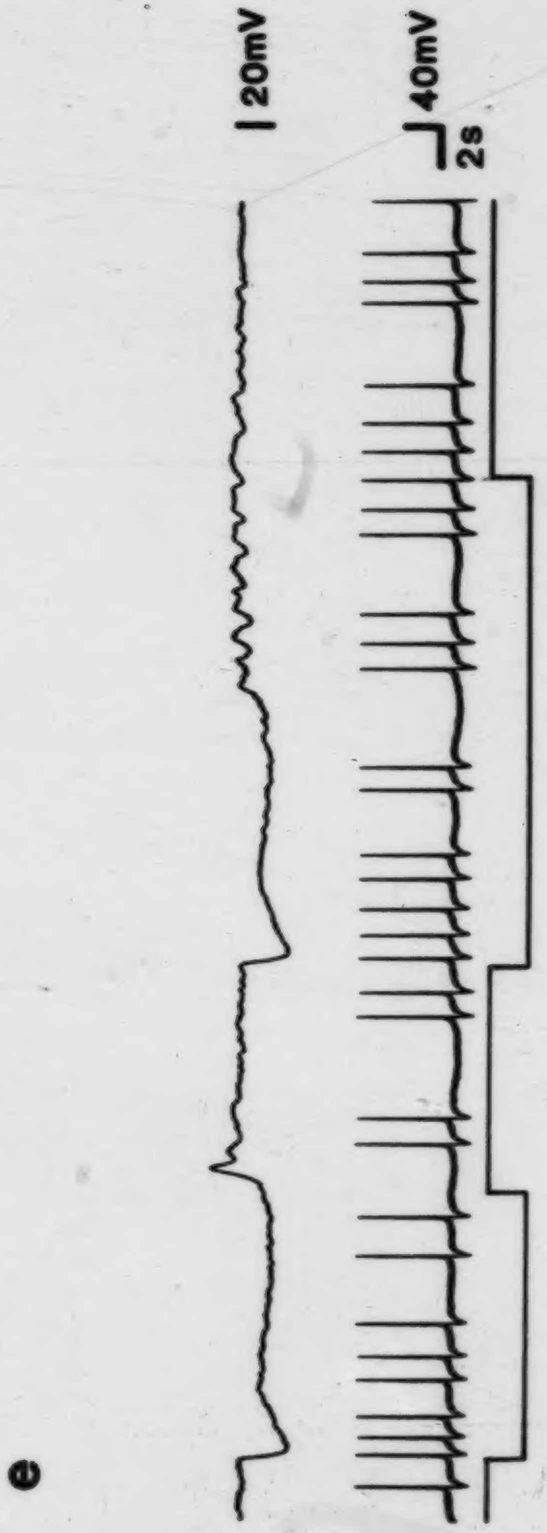

d
20mV
0.5mV
2s

Figure 20. e) Dual intracelllular recording from "O" system (top) and SMNs (bottom) from a preparation bathed in 1:1 mixture of isotonic $MgCl_2$ and sea water. Notice that the oscillations are severly attenuated in the "O" system, but it responds to the shadow as the "intact" "O" system. SMNs do not show any change in frequency or membrane potential.

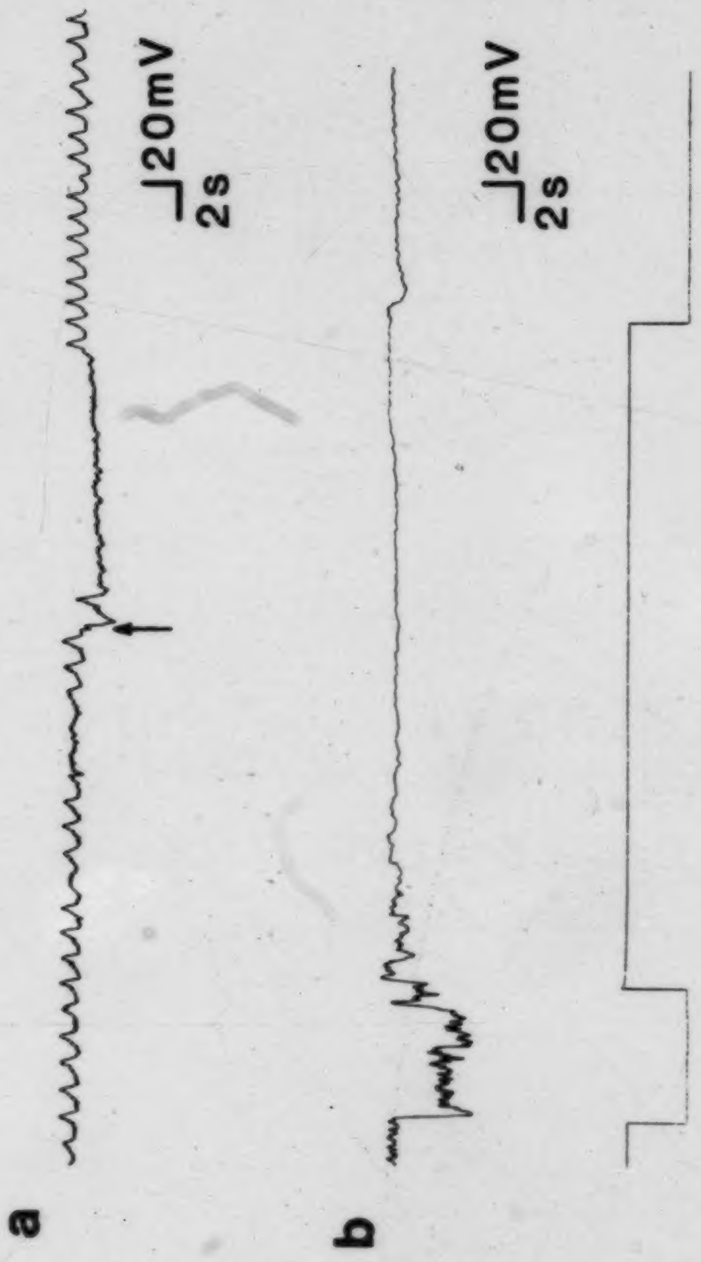

e
20mV
40mV
2s

system has adapted, there is little effect observed when the light intensity is increased.

Recording of the "O" system from the Ocellus

Because portions of the "O" system have been found to extend up to each ocellus (Spencer and Arkett 1984; Grimmelikhuijzen and Spencer 1984), attempts were made to record from the "O" system at the ocellar cup. Typical "O" system activity and responses to light intensity changes can be recorded from deep inside the ocellar cup (Fig. 21a). Only three such recordings were made after numerous attempts. This is probably due to the unstable nature of the recording location as local tentacle and other muscle contractions dislodge the electrode. Typical shadow responses of the "O" system have also been recorded while under $MgCl_2$ anesthesia (Fig. 21b) and they are comparable to those of the "O" system recorded in the ONR in $MgCl_2$ (Fig. 20e). The exact depth of the recording site was not determined, but it was below the open cup area.

Figure 21. a) Intracellular recording from deep within an ocellar cup of *Polyorchis*. Recording from an "intact" animal showing the response to shadows which is nearly identical to that of the "O" system recorded in the ONR. At light OFF (arrow), the membrane potential hyperpolarizes, the oscillations cease, and then reappear in the dark. b) Recording from the ocellus from an animal in 50% $MgCl_2$. The response is similar to that of the "O" system recorded in the ONR under $MgCl_2$.


a
20mV
2s
b
20mV
2s

Discussion

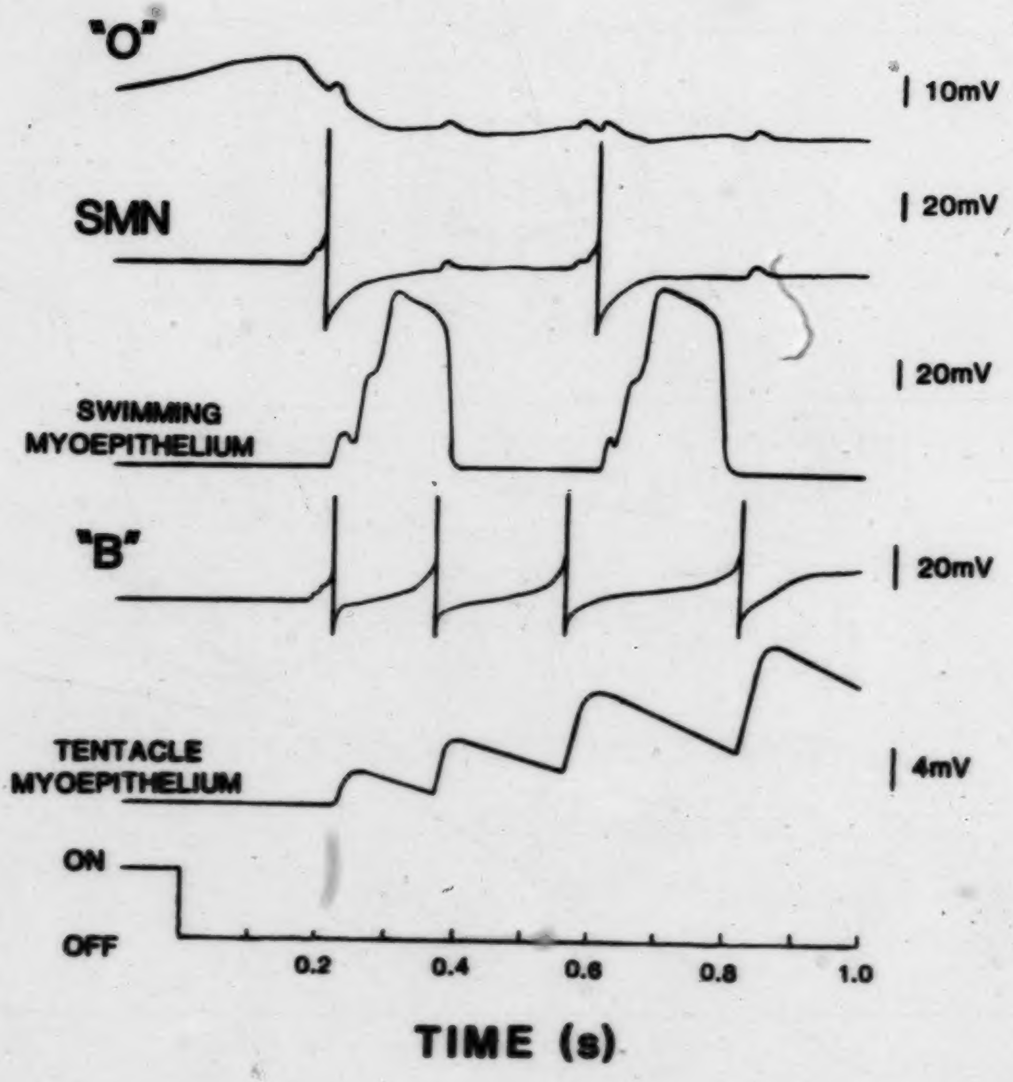
I suggest here that the simple behavior of ***Polyorchis*** in response to rapid reductions in light intensity is a simple reflex. If it is in fact a genuine reflex, then this behavior must meet the three criteria I have set forth. I will discuss here the first two of these criteria and present evidence for the third in Chapter 5.

Shadow Response Sequence

The response of ***Polyorchis*** to rapid reductions in light intensity involves several systems operating in a predictable and ordered sequence. Since it has not been possible to record intracellularly from all systems simultaneously, I have summarized the results from this and other studies to illustrate the suspected sequence of events during the shadow response (Fig. 22). The first detectable event seen after light OFF is the nearly simultaneous "O" system hyperpolarization and SMN depolarization after approximately 155ms; the "B" system initial depolarization lags slightly, by approximately 10 ms. This slight delay in the "B" system results in a similar delay in initiation of the first action potential, which typically occurs after the first SMN spike. Therefore, the barrage of EPSPs observed in the SMNs during the initial depolarization of the shadow response are not a result of "B" system spikes as is regularly seen during normal swimming (Spencer and Arkett 1984). Anderson and Mackie (1977) found similar results and suggested that another system, other than the "B" system (then known extracellularly as the marginal pulse or MP system) must be responsible for the EPSPs and depolarization of the SMN after a shadow. The "other" system may be the "O" system as the SMN and "O" system show nearly simultaneous changes in membrane potential. At present, it is not known whether the connections between the "O" system and SMNs and "B" system are mono- or polysynaptic, but there is no indication of any other neuronal system.

The first SMN spike produces EPSPs in the subumbrellar epithelial cells overlying the SMNs (approximately 3.0 ms latency) which leads to a biphasic action potential of the swimming muscles (Spencer 1982) (Fig. 22). The biphasic swimming contraction, which is seen only while recording close to the SMNs, can be broken down into an initial velar

Figure 22. Reconstructed intracellular recordings from the functional units of the shadow reflex in *Polyorchis penicillatus* showing the timing of each event after a shadow. Information for swimming myoepithelium is from Spencer (1982). The first event after the shadow is the hyperpolarization of the "O" system followed by the depolarization of the SMNs and "B" system. Notice that the "B" system fires at a greater frequency than the SMNs and results in rapid, summing tentacle contraction; this occurs before the second swimming contraction. Notice also that the first swimming myoepithelium action potential is shorter in duration than the second. EPSPs which result from feedback from the "B" system and SMNs can be seen in the "O" system.


"O"
10mV
SMN
20mV
SWIMMING MYOEPITHELIUM
20mV
"B"
20mV
TENTACLE MYOEPITHELIUM
4mV
ON
OFF
0.2
0.4
0.6
0.8
1.0
TIME (s)

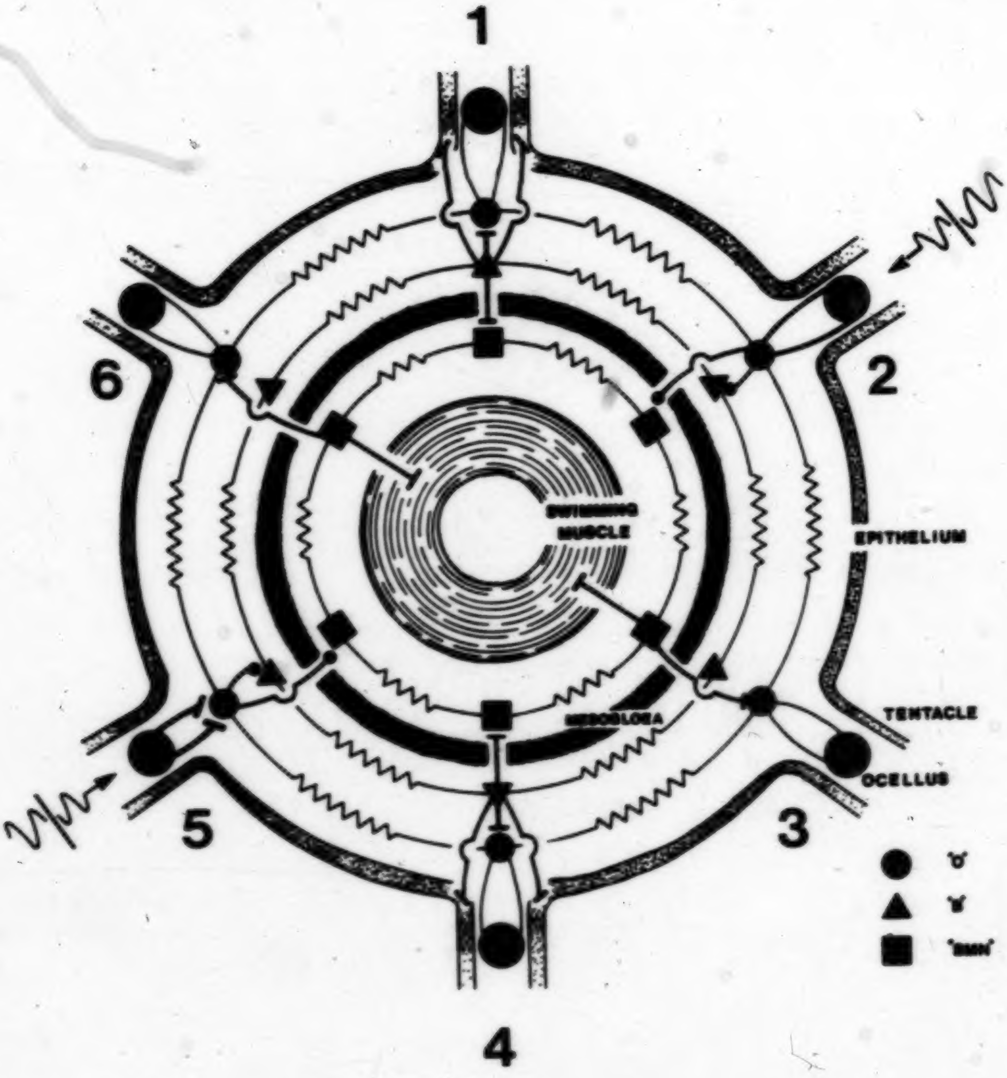
muscle contraction followed by sustained contraction of the subumbrellar swimming muscle which is observed in most medusae (Satterlie and Spencer 1983). Results shown in Figure 14 indicate a lower limit to the interspike interval of the SMNs at 400 ms, although there is some indication from behavioral observations that this apparent spike train adaptation is size-related as very small individuals show shorter interspike intervals and can therefore swim at higher frequencies (Fig. 7, Chapter 3). The spike train adaptation of the SMNs may be important in producing efficient swimming contractions by ensuring sufficient diastole before the next swimming contraction. Spencer (1978) found the swimming muscles were refractory until about 100 ms after their complete repolarization. Though swimming muscle contractions may sometimes begin before recovery has been completed (Gladfelter 1972), this is not normally the case because efficient jet propulsion requires a period of pump refilling before the next swimming contraction occurs (Gladfelter 1972; Spencer and Satterlie 1981; Daniel 1983).

The first "B" spike produces an EPSP in the tentacle myoepithelium after or concurrently with the first swimming muscle contraction, but due to the slow decay of the EPSPs and the short "B" system refractory period (Fig. 14), the high frequency burst causes rapid summation of subsequent EPSPs. The bursting characteristic of the "B" system ensures that the tentacles are fully contracted before the next swimming contraction. Thus, the behavioral sequence of events in response to a rapid shadow is highly predictable and consists of an initial single swimming contraction, followed by a series of rapid successive tentacle contractions, finishing with several more swimming contractions.

Reflex Arc Components

The predictable response of *Polyorchis* to shadows follows identifiable neuronal pathways and may involve all the components of a true reflex arc. Such an arc should include receptors, afferents, a centralized integrator, motor neurons, and effector organs. The connections and arrangements of these components are summarized in Figure 23. The motor and effector portions of the reflex are well understood from previous studies. SMNs control swimming muscle contractions (Spencer and Satterlie 1980) while the "B" system is clearly responsible for driving tentacle contractions. No such motor role is

Figure 23. Circuit diagram of the connections and components involved in the shadow reflex in *Polyorchis*. Resistor symbols represent electrical coupling between cells within each system. The "O" system is shown both as continuous with receptor cells of the ocelli (2)and as second-order neurons to the receptor cells of the ocelli (5) (see text for discussion on these differences). Both situations show inhibitory input onto the SMNs and "B" system and removal of this inhibition during a shadow may cause SMN and "B" system depolarization. Connections for the SMNs and swimming muscles (3, 6) are from Spencer (1978). Notice that the SMNs have excitatory feedback onto the "O" system. The "B" system (1, 4) has excitatory input onto the SMNs, "O" system, and epithelium. Excitatory input onto "epithelium" represents both ONR epithelium and tentacle myoepithelium. "Mesoglea" respresents the mesoglea that separates the inner and outer nerve-ring.


1
2
3
4
5
6
SWIMMING
MUSCLE
EPITHELIUM
TENTACLE
OCELLUS
MESOGLOEA
'O'
'B'
'SMN'

suggested by the "O" system as its regular, non-spiking oscillations do not correlate with any overt behavior. The central location of the "O" system between the ocelli and the swimming motor neurons suggests that it is primarily involved in the integration and afferent conduction of photic information (Chapter 5), but it may also be important in the detection of light.

Although the "O" system shows strong chemical synaptic feedback from the SMN in the form of discrete EPSPs (Fig. 20), it is as yet unknown how the SMNs "read" the rapid hyperpolarization of the "O" system at light OFF. The resultant depolarization of the SMNs could be a result of a shadow-induced release from "O" system inhibition. If an inhibitory transmitter were tonically released in light, "B" system and SMN activity would be suppressed. At light OFF, the "O" system hyperpolarizes, possibly causing a rapid release from inhibition of the SMNs and "B" system, both of which rapidly depolarize and spike. The rapid burst of spikes may be due to increased excitability caused by post-inhibitory rebound. That this process may occur is evidenced by the fact that the spiking frequency of the SMNs are graded with respect to the degree of inhibitory release (i.e., rate and magnitude of "O" system hyperpolarization , Chapter 5). Further evidence for this is given by Spencer (1981), who was able to inhibit SMN action potentials by injecting a *constant* hyperpolarizing current. However, the characteristic oscillations of the "O" system probably cause alternating inhibition and release of inhibition, the effect of which is occasional membrane depolarizations and spiking in the SMNs and "B" system. Only when the "O" system remains hyperpolarized (e.g., during extended light OFF periods, Fig. 20) does one see prolonged hyperpolarization of the SMNs and "B" system and an inhibition of action potentials. A similar mechanism for the bursting activity of the second-order neurons of the supra-oesophageal ganglion has been suggested to explain the barnacle shadow reflex (Milliecchia and Gwilliam 1972). In this system, GABA functions as the inhibitory transmitter which is tonically released from the barnacle receptor axon in light (Koike 1983). At light OFF, GABA release is suppressed and spiking activity of the supra-oesphageal ganglion is produced by a release from inhibition (Millecchia and Gwilliam 1972). This phenomenon of disinhibition, a suppression of an inhibitory transmitter release upon light increase or decrease, appears to be a common feature of photoreceptor systems in both vertebrates and invertebrates (Fain

1981; Laughlin 1981; Fain et al. 1983.). This same mechanism may function in *Polyorchis* but, unfortunately, there are no conclusive studies as to the nature of the transmitter of the "O" system (but see Grimmelikhuijzen and Spencer 1984). Attempts to rapidly hyperpolarize the "O" system by current injection to produce a burst of APs from both the SMNs and the "B" system have been unsuccessful, although the frequency of "O" system oscillations can be altered. Constant depolarizing current injected into the "O" system does increase the frequency of ocillations while constant hyperpolarizing current does stop oscillations. Loading of the "O" system due to its extensive electrical coupling probably prevents ring-wide hyperpolarization and prevents the bursting of the "B" system and SMNs.

The efferent feedback from the SMNs onto the "O" system (Fig. 20) may be important in altering the sensitivity of the photoreceptor system to light intensity changes. Kaplan and Barlow (1980) and Barlow (1983) found that efferent activity, which increases at night through a circadian clock, can drastically increase the sensitivity of *Limulus* lateral eyes. It was suggested that this increased sensitivity of nighttime "vision" is important for locating mates at night (Barlow 1983). Similarly, the SMNs may "prime" the shadow response by depolarizing the "O" system, restart the oscillations, thereby increasing the photic sensitivity during dusk. This property may be important in enabling the shadow response to recur repeatedly when light intensity is very low and the rate of decrease is greatest. This may explain how *Polyorchis* sees only the greatest rate of change in light intensity which occurs after sunset (Chapter 3). I have found no indication of a circadian clock system with respect to swimming activity as observations of individuals on the treadmill over 24 hour periods of constant light intensity shows no difference in swimming frequency. However, both the "B" system and SMNs do show endogenous spiking activity when isolated in $MgCl_2$ (Fig. 13 & 15). Long term recordings from these systems were not possible, but the "B" system at least may show some circadian activity. The function of the characteristic "O" system oscillations remain enigmatic. It is not clear if the post-synaptic systems "read" these oscillations or whether they are merely a function of excitatory feedback from the SMNs and "B" system and are unused (but see Chapter 5).

Even though the peripheral projections of the "O" system extending up each tentacle (Spencer and Arkett 1984) and the ocellar nerve (Spencer 1979; Singla and Weber 1982a) appear to be synonomous, some doubt remains as to whether the "O" system/ ocellar nerves are second-order neurons to the receptor cells of the ocellus or the receptor cells are terminal outgrowths of the "O" system. Several lines of evidence point to the latter explanation. The "O" system responds to shadows by graded potential changes (Chapter 5), typical for most primary photoreceptors, although second order neurons in both vertebrate and invertebrate visual systems also show graded potential changes (Fain 1981; Laughlin 1981). The "O" system shows these graded potential changes with or without the ocelli present and after isolation from synaptic inputs. The number of photoreceptor cells and axons in the ocellar nerve show a 1:1 correlation (Singla and Weber 1982a). I have shown in this study that normal "O" system recordings can be made from the ocellar cup (Fig. 21). Grimmelikhuijzen and Spencer (1984) using an antibody to FMRFamide conjugated to fluorescein found that the ocellar nerve (part of the "O" system) terminated at the ocellus in typical "flask-shaped" sensory cells which are similar to those of most medusan photoreceptors (Singla 1974). However, they state that these cells terminated at the periphery of each ocellus and the photoreceptor cells lying within the ocellar cup were not immunoreactive. These data all suggest that neurons of the "O" system within the nerve-ring, the extensions up to the ocelli, and the terminations of the "O" system at the ocelli are continuous and are innately photosensitive. The apparent photosensitivity of putative neurons in the subtentacular region of a closely related hydromedusan *Spirocodon* (Ohtsu 1983) may be explained by a photosensitive system similar to the "O" system of *Polyorchis*. Likewise, the apparent photosensitivity of the swimming motor neurons in *Spirocodon* (Ohtsu 1983) like *Polyorchis* (Anderson and Mackie 1977) may be attributed to chemically mediated input from the innately photosensitive "O" system homologue because it is clear from this study that the SMNs are not photosensitive. The pronounced photic response of hydromedusae that lack specialized photoreceptors (e.g., *Aglantha, Stomotoca, Gonionemus*) may also be explained by an innately photosensitive "O" system homologue. However, Satterlie (1985) proposes another explanation to extraocular photosensitivity. He has described putative extraocular photoreceptors that are located just outside the ONR fiber tracts and suggest that these structures have excitatory inputs onto ONR networks. There is, however, at present no direct evidence to support the photosensitivity of these structures.

Singla and Weber (1982a), however, provide morphological evidence which suggests that the "O" system is made up of second order neurons to the receptor cells of the ocelli. They showed afferent and efferent synapses between the receptor cells in the ocellar cup and the proposed secondary neurons. This arrangement has been observed in several other medusae (Toh et al. 1979; Yamamoto and Yoshida 1980). However, these chemical synapses may be due to connections between the "O" and "B" system at the level of the ocelli. The best evidence for this comes from Grimmelikhuijzen and Spencer (1984) who found that connections between the ocellar nerve (part of the "O" system) and the "B" system were present near the ocellus, as well as near the tentacle base. It is clear from the present study that these connections at the ocellus must be intact for the bursting response of the "B" system as ablation of the ocelli eliminates this response. Furthermore, Figure 19 shows that there is a differential delay in the initiation of "B" system-generated EPSPs which also suggests that "B" and "O" system connections at the ocellus are present. Further evidence that the connections between the "B" and "O" systems must be intact at the ocellus to produce a shadow response is given by Hisada (1956). Using *Spirocodon*, he showed that an isolated tentacle with an ocellus intact showed a normal response to a shadow, namely rapid summing contraction of the tentacle. Some portion of the "O" system would still be intact and its response to a shadow could produce bursting in the "B" system.

Eakin (1968) has suggested that the cnidarian photoreceptor cells may be the origin of the ciliary line of photoreceptors, which culminates with the vertebrate retina. Because the structure of the receptor cells of the vertebrate retina and those of the ocelli of *Polyorchis* are based on cilia (Eakin and Westfall 1962; Singla and Weber 1982ab), one might predict that their cellular responses to light intensity changes may be similar and in general, most ciliary photoreceptors *do* tend to show the characteristic graded hyperpolarization with a light stimulus (McReynolds 1976). Conversely, most of the photoreceptors of the rhabdomeric line (Eakin 1968) generally tend to show a graded depolarization to light stimuli (Laughlin 1981). There are, however, a growing number of exceptions to these generalizations as more animals are examined, suggesting that Eakin's scheme for the dichotomous line of photoreceptor structural (and presumably functional) evolution does not hold. Indeed, Salvini-Plawen and Mayr (1977) have suggested that there may be at least forty different phyletic lines of photoreceptor differentiation of unmodified cells. It seems reasonable to assume that cellular responses of photoreceptors may also show some diversity or at least not be confined to the cilium / hyperpolarization and rhabdom / depolarization dogma. Thus, we may not be able to make predictions about a photoreceptor response based solely on it morphology. I have proposed that the ciliary receptor cells of the ocellus are continuous with the "O" system and that it is the primary photoreceptor system. I will demonstrate that the "O" system shows a graded depolarization in response to light stimuli (Chapter 5), which is similar to the response of rhabdomeric photoreceptors. This apparent structure / function anomaly can not be resolved until it is shown that the "O" system *is* the primary photoreceptor and more intracellular information from other medusan photoreceptors is available. For this reason, I have included the two possible arrangements between the ocelli and the "O" system in the circuit diagram of the shadow reflex (Fig. 23).

From a behavioral standpoint, it may not be necessary to functionally differentiate the "O" system from the receptor cells. What is important is that at some predictable time after a shadow, a sequence of events is initiated that starts with the hyperpolarization of the "O" system. This, together with the centralized location of the "O" system in the ONR makes it a focal point for studying how photic information is integrated. Thus, the "O" system is of primary importance in the shadow response, possibly functioning as the receptor, certainly transmitting photic information to efferents which control tentacle and swimming muscle contraction, and as will be seen in Chapter 5, integrating photic information.

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V. ELECTROPHYSIOLOGY OF THE SHADOW RESPONSE II.

The Shadow Reflex of a Hydomedusan:

II. Possible Mechanisms of Light Integration and Functional Significance

Introduction

There are several general unifying properties of photoreceptors despite their wide variety of structural configurations. First, photoreceptors respond to changes in light intensity with a graded potential that is directly proportional to the magnitude of the change in light intensity. That this analog signal can convey very precise information about small changes in light intensity to post-synaptic systems is probably the most important function of the graded potential (Fain 1981). Secondly, many photoreceptors, both vertebrate (Fain et al. 1976; Schwartz 1976) and invertebrate (Laughlin 1981) are electrically-coupled. This property may function as a first line of photic integration by improving the detection of photic stimuli (Fain et al. 1976; Schwartz 1976; Gold 1979). It would, therefore, not be surprising that the well-developed photoreceptors of hydromedusae were electrically-coupled and showed graded potential changes in response to light stimuli. However, that an electrically-coupled photoreceptor system could function as a central integrator of photic information in the simplified nervous system of a hydromedusan has not been considered.

There are only a few studies that have suggested the presence of a graded electrical response of hydromedusan ocellar receptor cells to changes in light intensity. Weber (1982 a,b) recorded from the ocelli of *Polyorchis penicillatus* and *Sarsia tubulosa* and found a graded positive potential change in response to varying light intensities. Similar responses have also been suggested for *Spirocodon saltatrix* (Ohtsu 1983). However, these electroretinogram (ERG) studies used extracellular recordings from the ocelli and probably recorded from several different units. Thus, the responses they recorded are some combination of photoreceptors, second-order neurons (if present), and myoepithelium. Furthermore, their lack of identification of discrete systems precludes conclusions about the cellular nature of membrane potential changes of the photoreceptor system in response to light stimuli. Spencer and Arkett (1984) have shown that the electrically-coupled "O" system responds to a rapid change in light intensity. In Chapter 4, I have provided evidence demonstrating that the hyperpolarization of the "O" system is the first detectable event in the shadow response and that the "O" system is probably the primary photoreceptor system for the response. If the "O" system is the primary photoreceptor system, then intracellular recordings from this system should show the cellular properties of a hydromedusan photoreceptor, what specific stimuli are important to the shadow response, and how this photic information is integrated to produce the coordinated, predictable response.

I demonstrate here that the "O" system, which has been shown to be either the primary photoreceptors or second order neurons (Chapter 4), shows a graded response to the rate of change in light intensity. Intracellular recordings from the "O" system show that it hyperpolarizes at a rate directly proportional to the rate of light intensity decrease and that it depolarizes in proportion to the magnitude of light intensity increases. Spiking motor systems (SMNs and "B" system), which are post-synaptic to the "O" system, also show a graded response with the rate of depolarization and spiking frequency directly proportional to the same light stimuli. The graded responses of the various systems involved in the shadow response further supports my hypothesis that this response is a reflex as I had originally defined it (Chapter 4). I propose that the electrical-coupling properties of the "O" system are important for the integration of photic information in a radially symmetrical animal like a hydromedusan. I also provide further evidence that the shadow response does not function as a predator avoidance mechanism, but rather that slow shadows are important in initiating diel vertical migration.

Methods and Materials

Conventional intra- and extracellular recording techniques used in these experiments have been previously described (Spencer 1978; Spencer and Arkett 1984). All recordings from neuronal networks in the inner and outer nerve-rings of the hydromedusan *Polyorchis penicillatus* were made in seawater (15-18 °C). Medium-sized individuals with bell heights of 1-3 cm collected from Bamfield Inlet, Bamfield, B.C. were routinely used.

The light stimulus for these experiments was a green light emitting diode (Siemens LD57C) with a peak output at 560 nm. The LED was placed 1-2 cm from the outer nerve-ring and ocelli. Light intensity at full ON during routine recording conditions was approximately 2.0 μW / cm^2 s (measured by ISCO Model SR Spectroradiometer). This value is equivalent to about 0.10 microeinsteins / m^2 s. Various stimuli were produced by driving the LED with a function generator (Wavetek Model 180) through a current control circuit. "Instantaneous" light intensity changes were made by presenting square pulses of light with a square wave; slow changes in light intensity were made by using a triangular wave function. Percentage and absolute changes in light intensity were made by manually varying the DC offset and amplitude of the signal from the function generator. For square waves producing "instantaneous" changes, the duration of the switching time from one light intensity to another was 0.4 ms. Percentage change in light intensity is defined as $(I_0 - I_1) / I_0$ where I_0 is the initial light intensity and I_1 is the light intensity after some time (t). All experiments were done in a darkened room. No attempt was made to control adaptation times of the ocelli before experiments.

Results

Responses to "instantaneous" percentage changes in light intensity

Intracellular recordings from the "O" system show a graded response to varying "instantaneous" percentage changes in light intensity (Fig. 24). The response to reductions in light intensity is a rapid hyperpolarization of membrane potential occurring with the greatest magnitude in response to 100% reductions in light intensity. As the percent reduction in light intensity decreases, there is a corresponding decrease in the magnitude of the hyperpolarization. Percent reductions of light intensity less than about 28 - 30% fail to produce a detectable hyperpolarization. However, reductions less than 28% occasionally cause a slight decrease in the frequency and amplitude of the characteristic "O" system oscillations. In addition, for the greatest percentage decrease in light intensity, the "O" system remains hyperpolarized during the reduced light period. As the percent reduction decreases, the duration of the membrane hyperpolarization is shortened as it quickly adapts and returns to the resting potential. For increases in light intensity, the "O" system responds with a rapid depolarization and a resumption of the regular oscillations. The amplitude of the initial oscillation at light ON also shows a direct relationship to the percentage increase in light intensity (Fig. 24).

The SMNs also show a graded response to varying percentage changes in light intensity (Fig. 25). For reductions in light intensity, the depolarization amplitude and the number of action potentials is directly related to the percentage decrease in light intensity. One hundred percent reductions produce the greatest number of action potentials. For smaller percentage reductions, the membrane potential of the SMNs depolarizes only slightly, quickly adapts, and returns to the resting potential. The percentage decrease in light intensity at which no response could be detected was consistently around 26-28%, although very slight depolarizations have occasionally been observed with as little as 22% change. For increases in light intensity, the SMNs initially hyperpolarize. This response is most apparent for the largest percentage increases in light intensity and can often terminate a burst of action potentials. Hyperpolarization of the SMNs in response to the smallest percentage increases in light intensity were not detectable.

Figure 24. Intracellular recording from the ONR showing the "O" system response (top) to varying "instantaneous" percentage changes in light intensity (bottom). The magnitude of the hyperpolarization and depolarization is proportional to the percentage change in light intensity. Note that the membrane potential remains hyperpolarized only for the 100% reduction in light intensity. Current monitor reflects percentage change in light intensity. Full up light is ON; full down light is OFF. Time from ON to OFF is 0.4 ms. The absolute change in light intensity from ON to OFF is about 2.0 $\mu W / m^2$ [illegible]

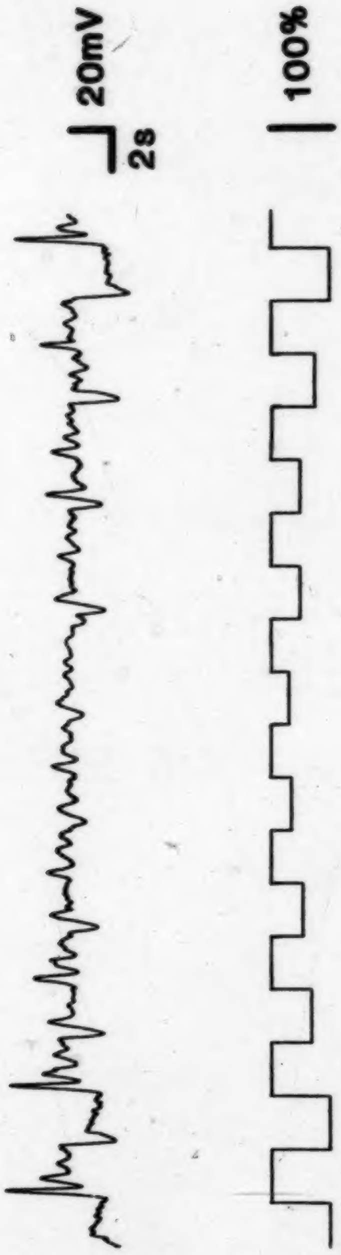
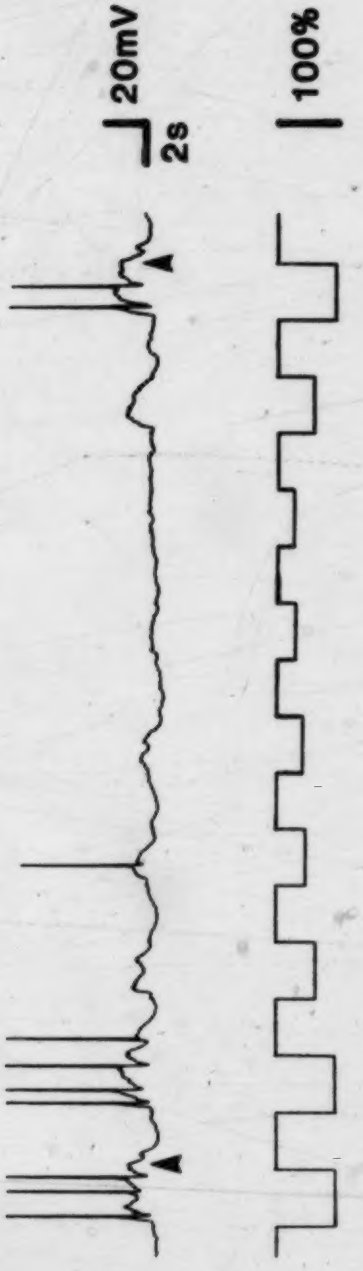

20mV
2s
100%

Figure 25. Intracellular recording from INR showing the SMN response (top) to varying "instantaneous" percentage changes in light intensity (bottom). The greatest number of spikes in a burst is present during 100% shadows while no response is seen to either increases or decreases to the smallest percentage change. Note that a burst of APs is terminated by an increase in light intensity (arrows).


20mV
2s
100%

The "B" system shows a similar response to these changes in light intensity (Fig. 26), although the hyperpolarization at light ON is not as pronounced as in the SMNs. Only a few of these recordings were possible and I was therefore unable to determine the threshold percentage change in light intensity.

Responses to "instantaneous" 100% changes at various absolute light intensities

The "O" system shows a nearly constant response to "instantaneous" 100% changes in light intensity at all the absolute light levels tested (Fig. 27). For 100% decreases in light intensity, the "O" system rapidly hyperpolarizes at light OFF with the membrane potential remaining hyperpolarized for the duration of the light OFF period. The membrane potential may adapt and return to resting potential after several seconds if the light remains off (Chapter 4). The delay from light OFF to the first detectable hyperpolarization is constant and does not vary with the absolute light intensities tested. During hyperpolarization at light OFF, the characteristic oscillations are severely attenuated, but high frequency PSPs are prominent. For increases in light intensity, the "O" system depolarizes rapidly and the oscillations resume their normal frequency and amplitude. Neither the frequency nor amplitude of the oscillations showed any correlation with the absolute light intensity during light ON periods, except at extremely low light intensities (Fig. 27). The "O" system does not appear to habituate to repetitive changes in light intensity as it shows nearly identical responses to 100% changes regardless of the number of consecutive repetitions.

The SMNs also show nearly identical responses to 100% changes in light intensity at varying absolute light intensities (Fig. 28). For decreasing light intensities, the SMNs respond with a rapid depolarization, with a constant delay, and fire a burst of action potentials. The number of action potentials and the amplitude of the underlying slow depolarization at light OFF is nearly identical for all the light intensities tested except at very low light intensities where spikes sometimes failed (Fig. 28). In situations where only a few APs are produced at light OFF, prominent, high frequency EPSPs are present on the slow depolarization (Fig. 28b). These are absent during light ON periods. The duration of SMN depolarization depends upon the duration of the light OFF period (Fig. 29). However, if the light OFF period extends for several seconds, then the SMN burst

Figure 26. Intracellular recording from the ONR showing the "B" system response (top) to varying "instantaneous" percentage changes in light intensity (bottom). The "B" system response appears to habituate quite rapidly to repetive stimuli.

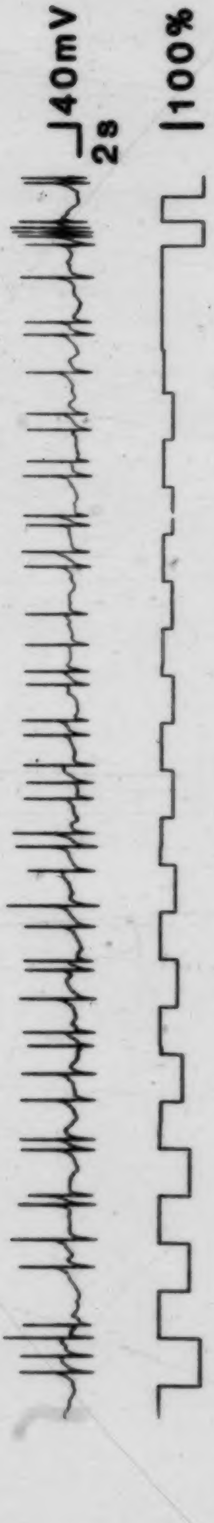

40mV
2s
100%

Figure 27. "O" system response (top) to 100% "instantaneous" shadows of varying absolute light intensities. Note that the OFF response is similar regardless of absolute magnitude of light intensity change. Shadow #5 appears to show some irregularity in its frequency of oscillations, which may be due to the low light intensity, but it does not appear to be much different from #10. Notice also that the "O" system does not habituate as the response to repetitive shadows is the same regardless of the number of consecutive trials. The occasional transient depolarizations during light OFF hyperpolarizations (arrows) are probably due to excitatory input from the SMNs. The mean (±1 SE) (n=11) delay from light OFF to the start of hyperpolarization is constant at 159.1 (6.1) ms for this trial. Light intensity at ON is approximately 2.0 μW / cm^2 s.

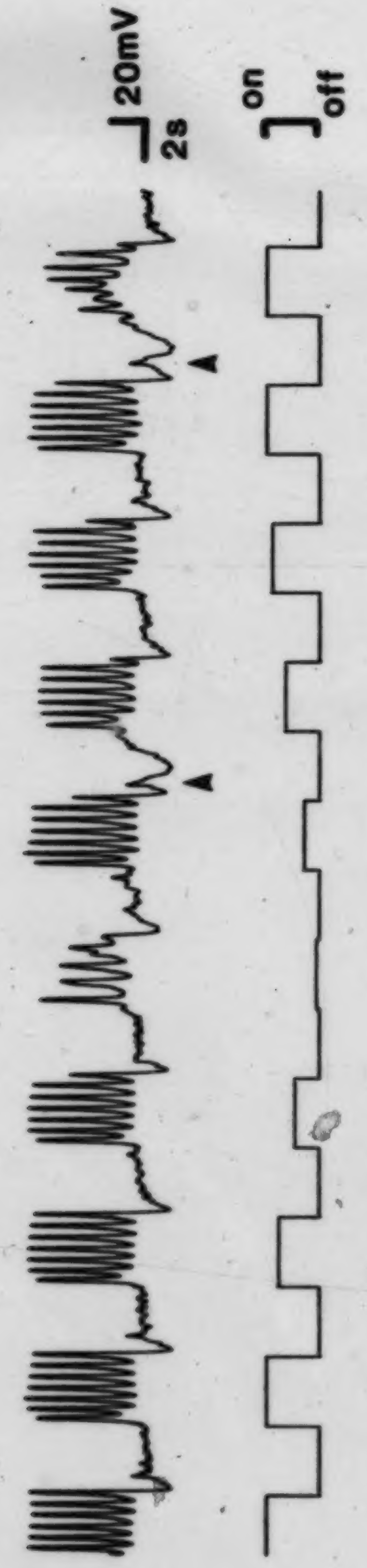
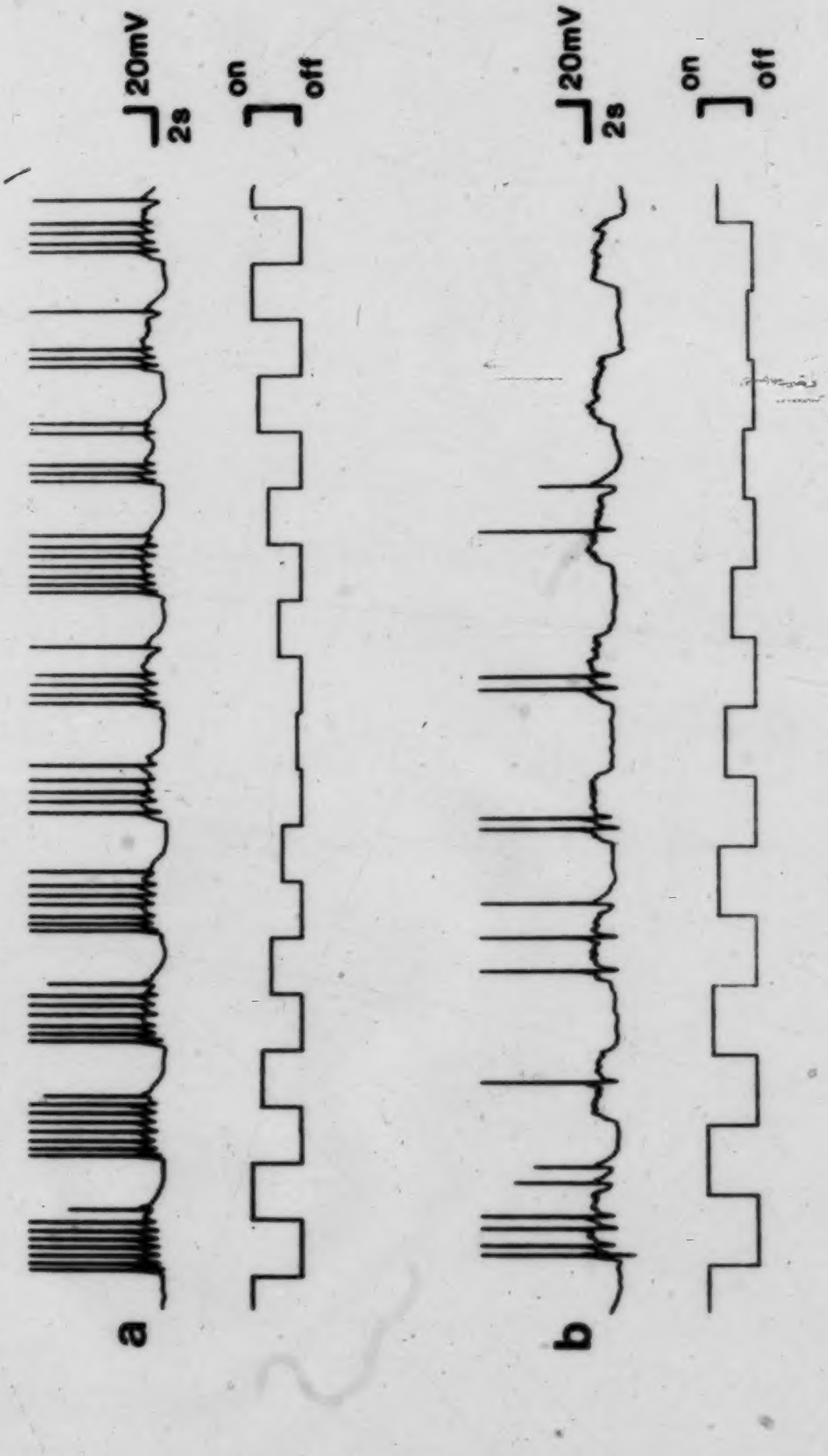

20mV
2s
on
off

Figure 28. SMN response to 100% "instantaneous" shadows of varying absolute light intensities. a) Light OFF produces a series of APs underlain by a slow depolarization of nearly the same magnitude regardless of light level. Increases in light intensity cause a hyperpolarization and often arrest the burst of APs produced by light OFF. Notice that SMNs do not appear to habituate to the 100% shadows and they will continue to respond indefinitely. The mean (±1 SE) delay from OFF to initial depolarization is 186.5 ± 5.9; n=10. b) A different preparation from (a) showing the response of SMNs to 100% shadows. Note the high frequency PSP activity during light OFF periods and their absence during light ON. The last two shadows caused marked depolarization of the SMNs with prominent EPSPs, however, the SMNs failed to spike. This may be due to the low light intensity as with the "O" system (Fig. 27).


a
20mV
2s
on
off
b
20mV
2s
on
off

slows and stops (Chapter 4). Increases in light intensity produce a hyperpolarization of the SMNs, often terminating a burst of action potentials. The membrane potential remains hyperpolarized throughout light ON period, but will begin to depolarize if light remains ON for several seconds (Chapter 4). The SMNs response to repetitive changes in light did not appear to habituate as they can follow frequencies of light change up to about 3 Hz (Fig. 29). Recordings from the "B" system for this experiment were not possible, but I suspect that they respond in a manner similar to the SMNs.

Responses to "slow" changes in light intensity

The "O" system shows a graded response to the rate of percentage decrease in light intensity. Figure 30a shows that as light intensity slowly decreases, the regular oscillations begin to decrease in amplitude and become progressively irregular as the membrane potential slowly hyperpolarizes. This gradual attenuation of oscillations and slow hyperpolarization is most noticeable during very slow reductions in light intensity (Fig. 30b). The rate of "O" system hyperpolarization is directly proportional to the rate of percentage light intensity decrease (Fig. 31). During light OFF periods, the membrane potential remains hyperpolarized until light intensity increases or the "O" system adapts and the oscillations spontaneously resume. The membrane potential depolarizes rapidly with the first light intensity increase and the oscillations return, usually with the initial oscillation larger and at greater frequency than subsequent ones. There does not appear to be a graded response to the rate of increasing light intensity, although the delay for depolarization is somewhat longer for slower increases in light intensity.

Intracellular recordings from the SMNs also show a graded response to gradual percentage changes in light intensity (Fig. 32). During slow reductions in light, the SMNs slowly depolarize under a barrage of EPSPs and begin to fire action potentials. Slower reductions in light intensity produce a more prolonged depolarization and greater number of APs, but at a lower frequency than faster reductions. During light OFF periods, high frequency EPSPs are prominent as the membrane potential remains depolarized. As light intensity gradually increases, the SMNs show a concurrent gradual hyperpolarization, which often interrupts a burst of action potentials. The high frequency EPSPs are absent during this hyperpolarization. Spencer (1981) and Spencer and Arkett (1984) noted that

Figure 29. Response of SMNs to 100% shadows at various frequencies of light stimuli. Note that the duration of the depolarization is directly related to the duration of light ON and that even for frequencies up to about 3.0 Hz, the SMNs respond although no action potentials are generated.

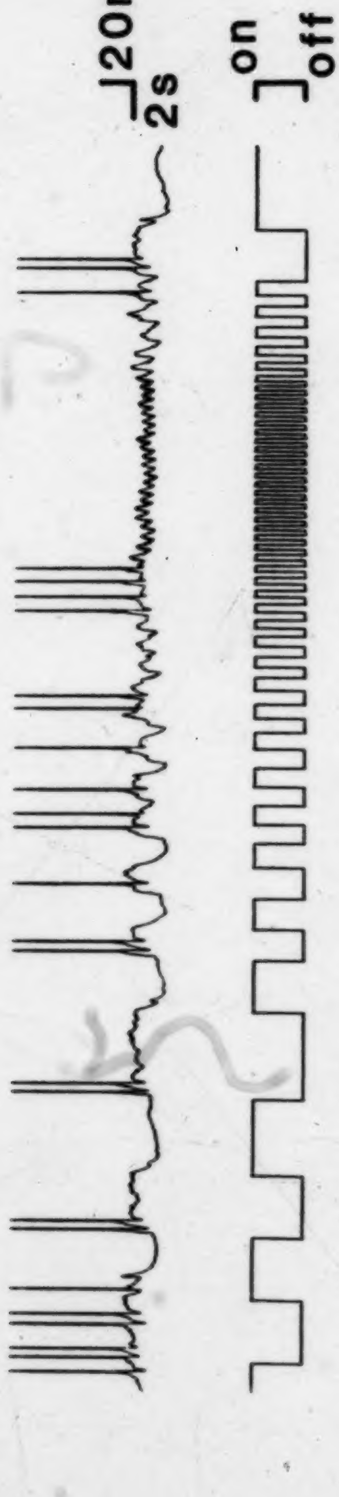

20mV
2s
on
off

Figure 30. a) "O" system response (top) to varying rates of percentage light intensity changes (bottom). Compare the slope of the "O" system hyperpolarization with the slope of the current monitor (light intensity change). Depolarization at light ON occurs with first light intensity increase and oscillations resume normal frequency. Initial oscillations are often of greater amplitude and slightly greater frequency than subsequent ones. Notice also that the system does not habituate to slow changes in light intensity. b) "O" system response to the slowest attempted rate of light intensity change (1.7% / s). Oscillations start to become very irregular and then the "O" system hyperpolarizes. Light is fully OFF at large arrow. Small arrows indicate possible unitary inhibitory post-synaptic potentials IPSPs that appear only during membrane hyperpolarization.

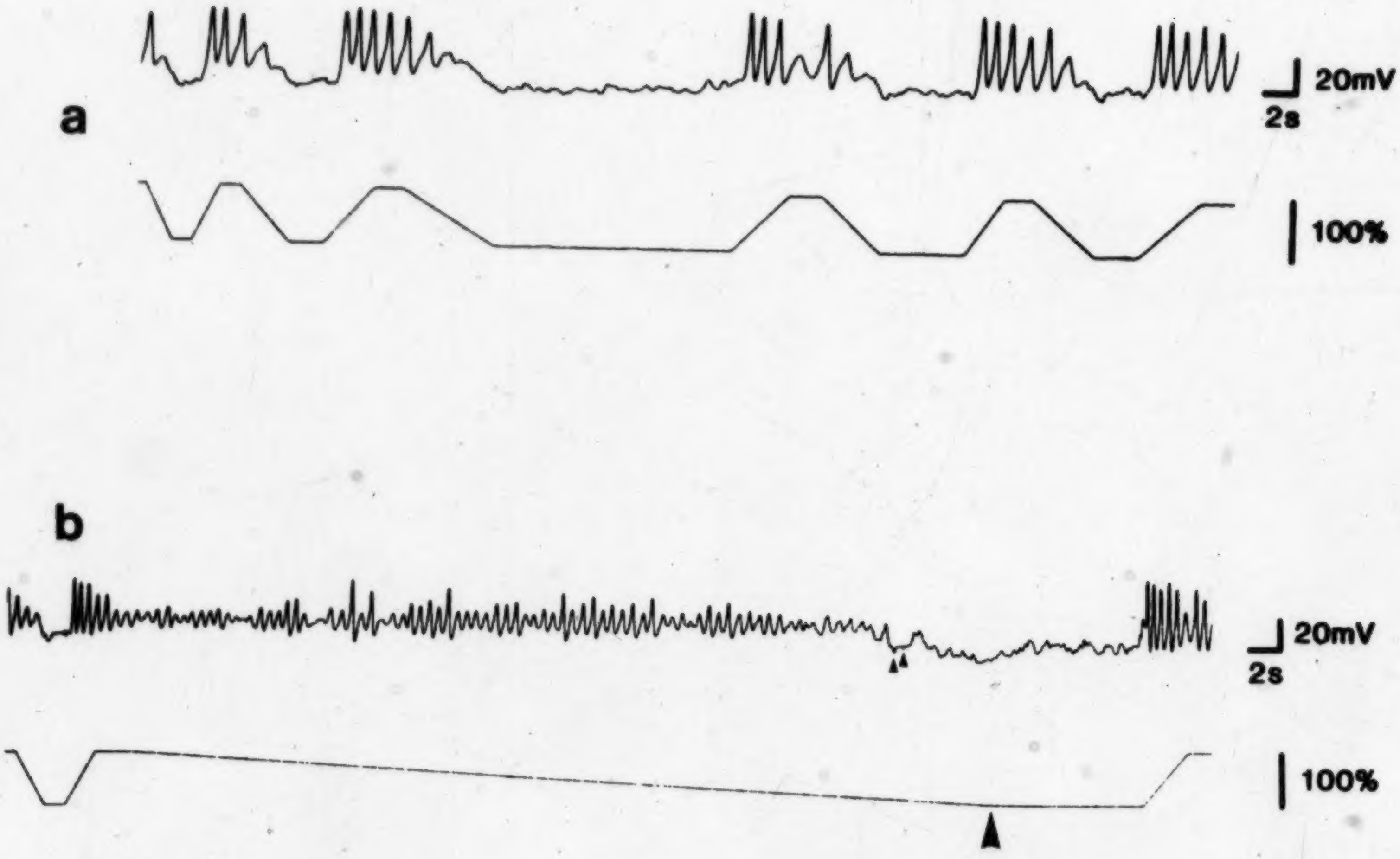

a
20mV
2s
100%
b
20mV
2s
100%

Figure 31. Plot showing the linear relationship between the rate of percentage change in light intensity and the slope of the "O" system hyperpolarization during slow changes in light intensity. Data were taken from intracellular traces from 2 different animals. $Y = 3.04 + 0.28 X$; $r = 0.94$; $n = 22$. Regression is significant at $0.01 > p > 0.001$. Rate of "O" system hyperpolarization appears to be limited because for "instantaneous" reductions (0.4 ms duration , 100% / 0.4 ms = 25,000% / 100 ms) the maximum rate of "O" system hyperpolarization was 20 mV / 100 ms.

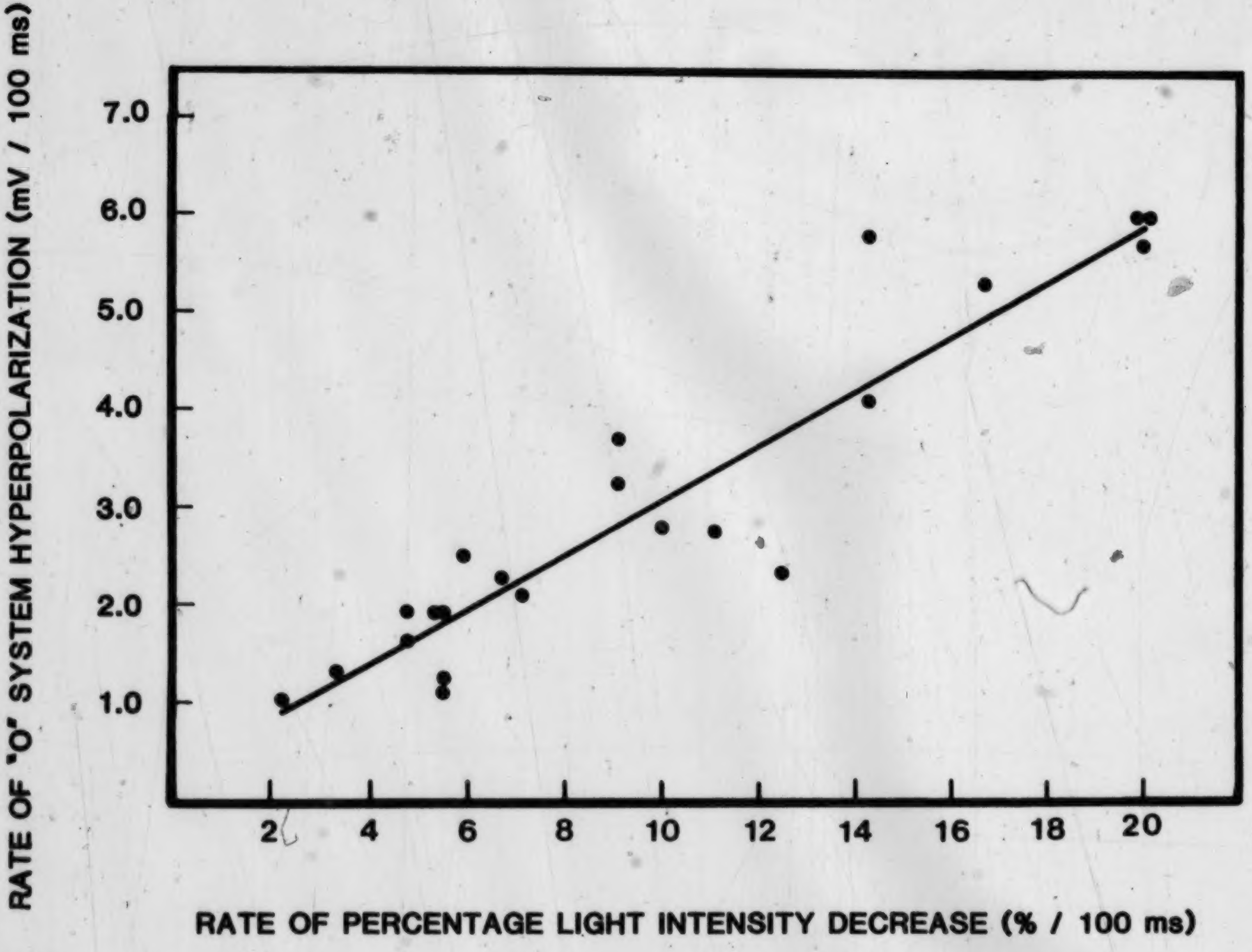
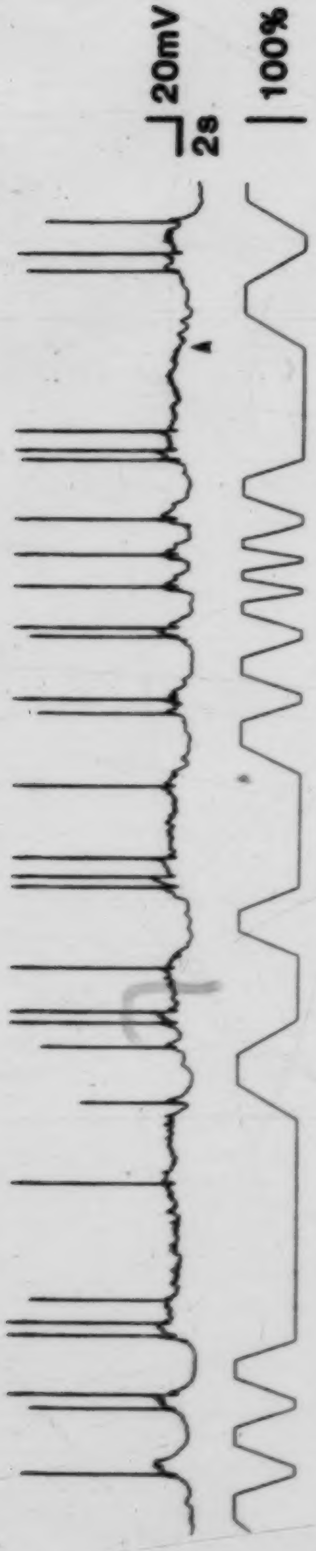

RATE OF "O" SYSTEM HYPERPOLARIZATION (mV / 100 ms)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
2
4
6
8
10
12
14
16
18
20
RATE OF PERCENTAGE LIGHT INTENSITY DECREASE (% / 100 ms)

Figure 32. Intracellular recording of SMN (top) showing its response to varying rates of percentage light intensity change (bottom). Note high frequency EPSPs during light OFF and underlying slow depolarizations during decreasing light intensity. As light intensity increases, EPSPs are immediately absent (arrow) and do not appear to be solely a function of membrane potential. Slow increasing light intensity again causes a progressive hyperpolarization. The response to repetitive reductions in light intensity does not appear to habituate.


20mV
2s
100%

spontaneous hyperpolarization of the SMNs can occur and are attributable to action potentials in the epithelium overlying the nerve-rings. However, the hyperpolarization of the SMNs during increases in light intensity occurs without any epithelial spiking activity (Fig. 33). The slow hyperpolarization of the "O" system and the depolarization of the SMNs are nearly in phase with the "O" system slightly leading the SMNs (Fig. 34). I was not able to record simultaneously from the "O" system and SMNs and give "instantaneous" shadows in order to determine conductance delays. However, data from Chapter 4 on delays from the various systems suggests that there are monosynaptic connections between the "O", "B" systems, and SMNs.

The "B" system shows a response similar to that of the SMNs, however, the hyperpolarization during light increases is not as pronounced (Fig. 35).

Figure 33. Intracellular recording of SMNs (top) with extracellular recording over ONR (middle) and LED current monitor (bottom). Note that the "O" (●) and "B" (▲) systems are active while SMNs hyperpolarize during light intensity increases. No spiking activity from epithelial cells, which would be seen as large, biphasic potentials in the extracellular trace, is present in the ONR recording during increasing light intensity and SMN hyperpolarization. Notice that the first two small depolarizations in the intracellular trace (arrows) which correspond to the "B" system spikes are larger than subsequent ones. This is often seen when the "B" system fires in pairs (Spencer and Arkett 1984).

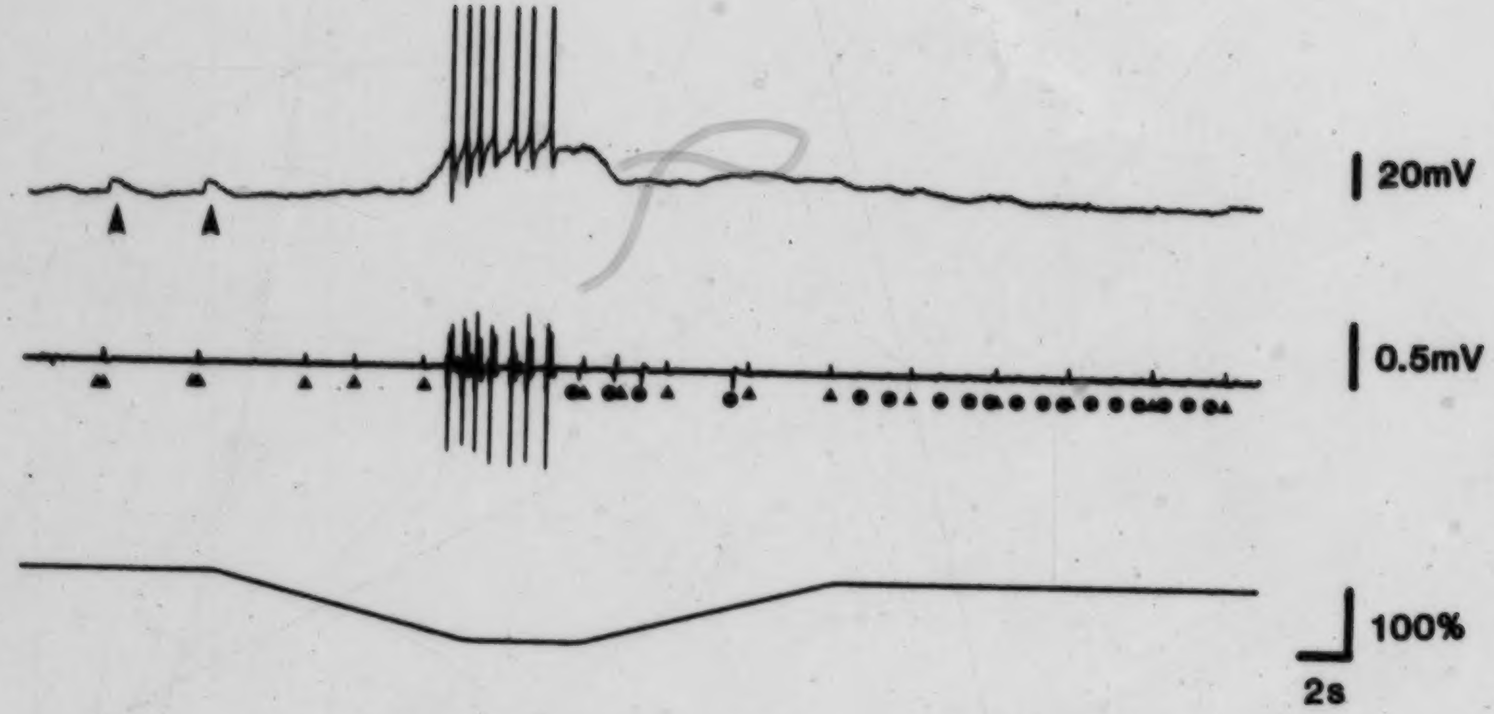

20mV
0.5mV
100%
2s

Figure 34. Simultaneous intracellular recording from the "O" system (top) and SMNs (middle) with LED current monitor (bottom) showing the responses during slow changes in light intensity. Notice the interpolation of small oscillations in the "O" system resulting from SMN spikes (arrows). SMN spikes feedback onto, depolarize, and speed recovery of the "O" system oscillations. Notice the high frequency EPSPs in the SMNs during decreasing light intensity and hyperpolarization of the "O" system. SMN spikes are "clipped" by the pen recorder amplifier.

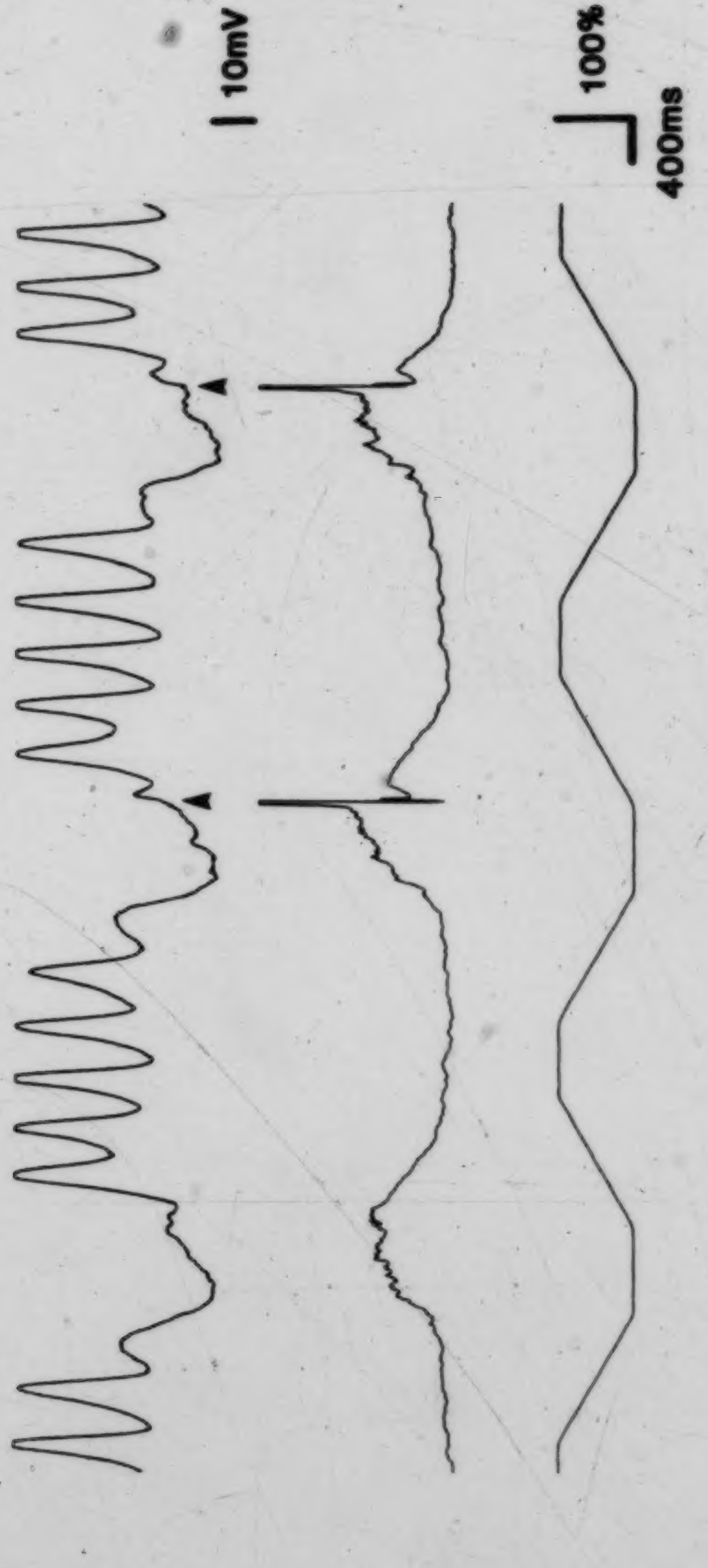
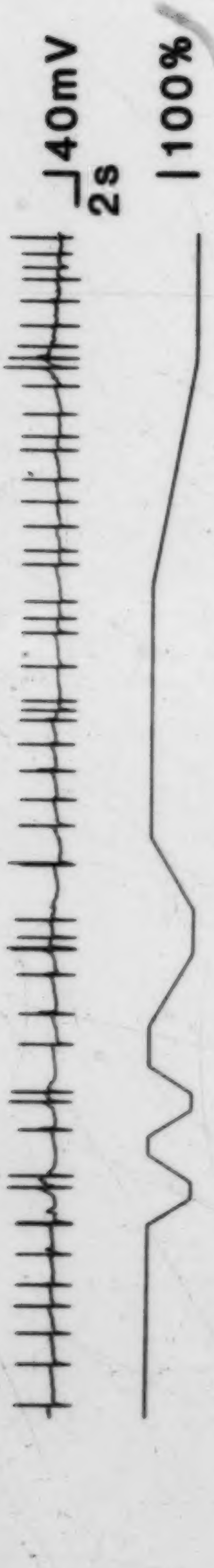

10mV
100%
400ms

Figure 35. Intracellular recording from the "B" system (top trace) showing its response to slow changes in light intensity as shown by the current monitor of the LED (bottom trace). Note the slow underlying depolarization during increasing spiking activity at light OFF.


40mV
2s
100%

Discussion

Graded response of the shadow reflex components

The graded nature of the "O" system's photic response demonstrated here appears to be similar to that found in the few hydromedusan ERG studies that have been done. Although it is difficult to compare the results from this chapter and Chapter 4 with these studies (primarily due to their lack of intracellular information), several comparisons can be made. Weber (1982a) conducted an extensive ERG-like study of the photic response of *Polyorchis*. While recording extracellularly from the ocellar cup, he found graded electrical activity in response to flashes of light of varying intensity. This positive deflection appeared after a 50-60 ms delay from the onset of light flashes. The "O" system shows a similar graded positive response to increases in light intensity (Fig. 24), although the delay found in my study is slightly greater. This difference in delay time could be due to synaptic delay between a primary photoreceptor and secondary neurons since Weber was recording at the ocellar cup and my recordings were in the ONR. This seems, however, far too long a period to be attributable solely to synaptic delay. Alternatively, this delay discrepancy may be an age-related problem since Weber used small medusae and "younger" ocelli. I have suggested in Chapter 3 that younger medusae may be more responsive than older medusae and this phenomonon may produce these delay differences. Although Weber's (1982a) ERG study, like most ERGs, are primarily interested in the response of photoreceptors to increases in light intensity, he showed that ***Polyorchis*** also responded to light OFF with a slow positive deflection with a latency of 250 ms, followed by high frequency pulses. I have shown a graded hyperpolarization of the "O" system at light OFF, the delay of which is nearly 100 ms faster. I have no good explanation for the discrepancy in polarity of these OFF responses. It seems unlikely that Weber's AC extracellular recordings could pick up the low frequency oscillations of the "O" system, except at light ON or OFF when the change in membrane potential is most rapid. The high frequency pulses following light OFF described by Weber (1982a), which he thought originated in the "nerve plexus of the optical nerve", are almost certainly due to either "B" system action potentials or tentacle myoepithelium contractions. It is difficult to compare the results of Weber's study with my results since the ERG recordings would include many components. Indeed, this problem has traditionally been the source of much confusion in cnidarian neurobiology. Weber (1982a) concedes that precise interpretation of his own results is difficult because his recordings "consist(s) of component responses recorded from photoreceptors, pigment cells, and second-order neurons of the optic nerve". Similar electrical events have been recorded in a closely related anthomedusan *Spirocodon saltatrix*. Ohtsu (1983) found that while recording in the ocellus (probably extracellularly), *Spirocodon* shows spontaneous oscillations in the dark. These oscillations appear to be analogous to the "O" system oscillations seen in *Polyorchis*, although the oscillations in *Spirocodon* are slightly smaller amplitude and slightly greater frequency. When the ocellus is illuminated with 500 nm light, there is a large initial rapid positive oscillation. At light OFF, "spike-like" activity superimposed on a slower positive potential was observed. This spike-like activity may again be attributable to a "B" system homologue and tentacle myoepithelium contractions. However, bearing in mind the differences in recording techniques and light stimuli, the similarities between the results of Weber (1982a) and Ohtsu (1983) and my findings support my interpretation that the "O" system is the functional photoreceptor system in *Polyorchis*.

An ERG from the ocellar cup of *Sarsia tubulosa* appears to show a similar response as that seen in *Polyorchis* and *Spirocodon* (Weber 1982b). However, a slightly delayed, opposite polarity deflection of similar time course, is seen while recording from the more proximal optic ganglion. This polarity change, which is not seen in *Polyorchis* or *Spirocodon*, is an enigma because one might expect such a change during transmission between receptor cells and second-order neurons. However, no second-order neurons are found in *Sarsia*, that is, the receptor cells of the ocelli are continuous with the ocellar nerve (Weber 1982b ; Singla and Weber 1982). Additionaly, Weber (1982b) found that recording from the tentacular ganglion near the ONR failed to show any activity directly correlated with the ERG. Based on Weber's findings, the photoreceptor organization and mechanism appears to differ from that of *Polyorchis* and *Spirocodon*. That the resulting photic behavior may also differ from that of *Polyorchis* and *Spirocodon* was shown by Romanes (1885). He demonstrated that *Sarsia* did not show a shadow response. In fact, Romanes (1885) showed that swimming in *Sarsia* is inhibited in the dark, but swims actively in the light.

The graded potential of the "O" system may be important in providing more precise information on very small, relatively slow changes in light intensity, a feat less likely with a digital signal. This fine tuning may be possible if the resting potential of a non-spiking system, like the "O" system, were within a few millivolts of the threshold for transmitter release (Wilson and Phillips 1983). Furthermore, if a non-spiking cell tonically releases transmitter, as photoreceptors cells generally do (Chapter 4) (Fain 1981; Laughlin 1981; Fain et al. 1983), then even a slight depolarization or hyperpolarization of the membrane potential could produce a graded increase or decrease in the amount of transmitter released. Thus, this analog mechanism has the effect of maximizing the sensitivity of the photoreceptor system. A similar mechanism may be operating with the graded, non-spiking "O" system , enabling it to respond to the smallest pre-synaptic voltage changes or to the smallest change in light intensity and thereby causing a graded release of transmitter to the post-synaptic systems (SMNs, "B" system). This graded release thus enables the shadow response to be a graded function of the rate of light intensity change.

I have demonstrated here the graded response of the non-spiking photosensitive "O" system and shown that the rate of hyperpolarization of the "O" system is directly proportional to the rate of decreasing light intensity (Fig. 31). In addition, the two post-synaptic spiking motor neuron systems involved in the shadow reflex (SMNs, "B" system) show at first a corresponding graded response to this same stimulus, which is subsequently coded into spiking frequency. Thus, I have demonstrated that the expression of the shadow response is a graded function and is directly related to a *specific* stimulus. This final component together with the demonstrable, predictable sequence of events and identifiable units (Chapter 4) conclusively establishes that the shadow response of *Polyorchis* is a reflex as I had initially defined it.

There is no *a priori* reason that the radially symmetrical nervous system of a hydromedusan must conform to bilaterally symmetrical neuronal concepts, such as a reflex. However, reference to and demonstration of the shadow response as a simple reflex has important connotations for the organization and mechanisms of simple nervous systems. The idea that medusae showed reflexive behavior is not novel. Indeed, early workers

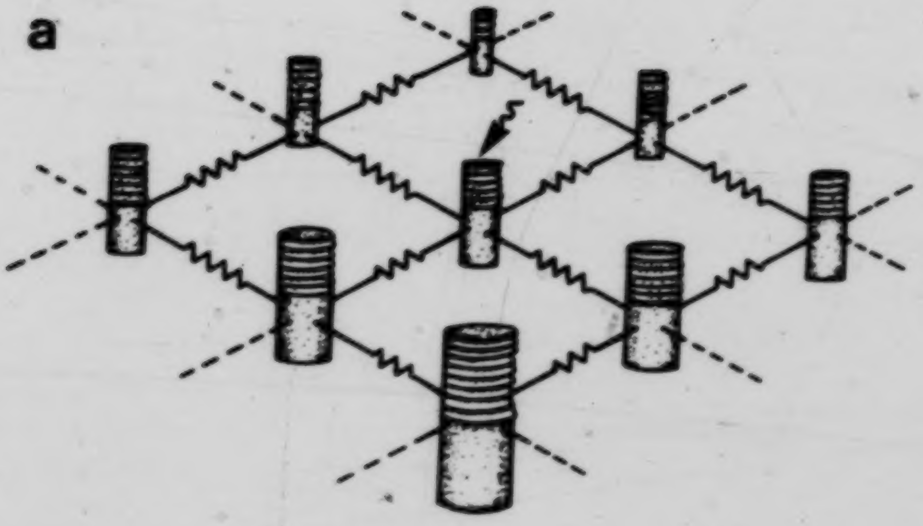
(Romanes 1885; Sherrington 1906) often used this term even though they labeled some behavior as a reflex solely on the graded behavioral response. I have also demonstrated the graded nature of the shadow response (Chapters 3 and 5). More importantly, however, I have been able to identify by intracellular recordings the various components involved in a specific response and to show the cellular properties and function of these identified systems. My proposition that the shadow reponse is a reflex suggests that some "centralized adjustor" must be present between the "receptor-effector" system characteristic of medusae (Parker 1919). I propose that the "O" system is centralized between the receptors and effectors and further, that it is involved in the integration of light information for the shadow reflex (see below) and thereby *functioning* as a simple central nervous system in *Polyorchis*. This is not to say that the "O" system is organized into some kind of "ganglion" as Mackie (1971) has suggested for *Sarsia*. But, the perceived lack of any kind of "centralized adjustor" in hydromedusae has created a belief that there is a concurrent lack of coordinated behavior, such as a reflex. Merely by using the work reflex to described some simple behavior, like the shadow response, we do not return to the idea that behavior is a result of a "chain of reflexes" (Sherrington 1906), but identification of reflexes will enable coelenterate neurobiologists to refer to functional neuronal components, to order behavioral hierarchies, and to suggest mechanisms of more complex behaviors.

Integration of Light by Electrically-Coupled Networks

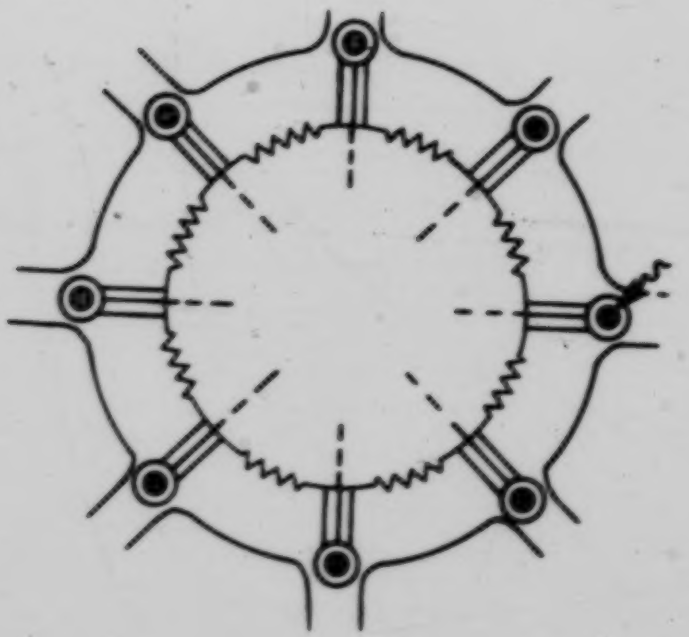
The electrical coupling properties of the "O" system (Spencer and Arkett 1984) may enable it to integrate information in a way analogous to the simple integration that takes place in the electrically-coupled receptor cells of the vertebrate retina (Fig. 36). The importance of the strong electrical coupling between receptor cells of the retina lies in the fact that low intensity light may be amplified and detected by spatial summation of inputs from widely spaced receptors (Baylor et al. 1971; Fain et al. 1976; Schwartz 1975, 1976; Gold 1979). In addition, the membrane potential changes in the receptors tend to be synchronized by rapid electrotonic conduction through gap junctions, leading to a temporal summation of inputs and enhancement of the signal. Extensive electrical coupling of the receptor cells and more proximal cells (e.g., horizontal, bipolar) may also

Figure 36. a) Schematic diagram of the planar array of electrically-coupled receptor cells in toad retina as proposed by Fain et al. (1976) and Gold (1979). b) Schematic of the electrically-coupled "O" system connecting the radial array of individual ocelli of *Polyorchis*. In each case the resistor symbol (-\/\/\/\/-)respresents the electrical coupling resistance between individual receptor cells or ocelli. Light stimuli impinging upon a single receptor cell (a) or ocellus (b) should not produce a response in respective post-synaptic cells, (horizontal, bipolar or "B" or SMNs). If light stimulates all receptors and all ocelli simultaneously, post-synaptic cells should respond due to the spatial and temporal summation of PSPs.

a



b



improve the signal-to-noise ratio by "averaging out" photic noise (Attwell et al. 1984). An analogous situation may occur in the wide, radial distribution of the ocelli through the strong electrical coupling of the "O" system. Because it is unlikely that individual receptor endings in a single ocellus would "see" different stimuli, I have considered the individual ocelli, which are connected by the electrically-coupled "O" system, to be comparable to the single, electrically-coupled rods in the model of Fain et al. (1976) and Gold (1979). Low intensity light or shadow information impinging upon several widely spaced ocelli should be synchronized by rapid conduction around the ring and summed spatially and temporally. Junctional shunting should be reduced resulting in an increase in the time constant of PSPs and temporal summation of subsequent PSPs. The amount of spatial and temporal summation of PSPs in the SMNs and "B" system would depend upon the number and distribution of ocelli stimulated, but at present it is unknown how many ocelli are required to "see" a shadow in order to produce a shadow response. This critical number of ocelli for the shadow response is probably not important during the changing light intensities at dusk and dawn because all of the ocelli would be stimulated to the same extent. That photic information may be amplified through the simultaneous stimulation of all ocelli may enable *Polyorchis* to detect the most rapid changes in the low light intensity that occurs after sunset and before sunrise (Chapter 3 and Munz and MacFarland 1973). It seems very unlikely that shadows which impinge upon only one or a few closely spaced ocelli would result in either localized tentacle contraction or a systemic shadow response. This is likely due to the difficulty in overcoming the large amount of shunting of localized light information through the electrically-coupled "O" system. This effect can be illustrated by Weber's (1982a) finding of no inter-ocellar interaction when he stimulated a single ocellus. Thus, through its extensive electrical coupling and inherent properties, the "O" system appears to function as an input filter and an integrator making *Polyorchis* unresponsive to local photic inputs, but very responsive to widely distributed , low intensity photic inputs from around the bell.

One way in which localized photic effects may occur is if there is localized uncoupling of the electrically-coupled "O" system. Several studies using electrically-coupled horizontal cells of turtle and fish have shown that some synaptic transmitters, which are known to be present (e.g., GABA, dopamine), can reduce the summation area of the receptive field by increasing the electrical-coupling resistance between the horizontal cells (Negishi et al. 1983; Piccolino et al. 1982, 1984). Whether a similar receptive field narrowing by localized uncoupling occurs in *Polyorchis* and if it has any functional significance is unknown. It is clear, however, that local contractions of the velum and tentacles can occur (Chapters 2 and 4), resulting in turning behavior (Chapter 2 and Gladfelter 1972) or selective tentacle contractions. These activities may be due to localized uncoupling of the myoepithelial cells, "B" system, or completely separate local pathways.

The highly predictable, ontogenetic changes in the number and position of ocelli in *Polyorchis* suggests that there is some plasticity in the resolution and the sensitivity of the shadow reponse. It is not known how the additional inputs, by way of additional ocelli, alters the electrical coupling properties of the "O" system, but more ocelli in older animals may increase the sensitivity of the shadow response. This may be important to the older individuals, which generally occupy a deeper position in the water column and "see" lower light intensities than younger individuals.

Functional Significance of the Shadow Response

Predator avoidance has often been invoked to explain the functional significance of shadow responses (Gwilliam 1963,1965; Singla 1974; Forward 1977). For a medusa, the short burst of swimming in response to a rapid shadow may result in movement sufficient to evade predators. For escape swimming to be effective, Daniel (1983) has shown that overcoming the "acceleration reaction" in the first few swimming cycles and once moving, reducing the drag, are important. The shadow response of *Polyorchis* appears, at first, to conform to these requirements as demonstrated by the sequence of events in Figure 22 (Chapter 4). The initial effector response to a rapid shadow is the short duration contraction of the swimming muscles, followed by slightly longer duration contractions (Spencer and Satterlie 1981). Efficient propulsion requires strong, long duration contraction of the swimming muscles. For this reason, the first contraction, which is relatively short in duration, may not be as effective as subsequent contractions in expelling water from the bell. Only when prolonged contractions occur should maximum velocity be attained, which is usually not until the second contraction (Fig. 22 and Gladfelter 1972).

However, the initial contraction may be important in overcoming the acceleration reaction (Daniel 1983). The rapid contraction of the tentacles after the first swimming contraction (Fig. 22, Chapter 4) may also be important in reducing drag, which Daniel (1983) showed to begin to increase after several contractions.

Although these findings tend to support the idea that the shadow response is used in rapid swimming, which presumably is used in escape movements, this behavior is short lived and does not result in large movements. The results of some of the experiments in this study corroborate those in Chapter 3. I have shown that the number of swimming contractions in the shadow response is directly related to the duration of the 100% shadow (Fig. 29). Furthermore, there appears to be a threshold of approximately 28-30% reduction in light intensity for the shadow response. Most predator- or wave-generated shadows seen by *Polyorchis* under field conditions would be much less than 100% and would probably be very short duration. These types of shadows would, as has been demonstrated, produce only 1-2 swimming contractions which would not alter the normal swimming frequency and thus, would not result in a significant escape distance. Furthermore, any swimming burst initiated by the shadow would be terminated by the inhibition of the SMNs during subsequent light intensity increases. However, as I have discussed in Chapter 3, significant distances may be travelled after a marked change in the "maintenance" swimming frequency (Chapter 2 and Arkett 1984). This change has been shown to be during slow , continuous changes in light intensity (Fig. 10 Chapter 3). Here I have demonstrated that the SMNs show a graded depolarization and proportional spiking frequency in response to slow reductions in light intensity, which correlate with the graded hyperpolarization of the "O" system (Fig. 30 and 31). Furthermore, slow increases in light intensity is correlated with gradual hyperpolarization of the SMNs. This explains the inhibition of swimming during increasing light intensity in the treadmill experiments (Fig. 10 Chapter 3). These slow changes in light intensity would not be produced by a predator, but more likely result from changing light intensity conditions at sunrise and sunset. Thus, the shadow response is more likely used by *Polyorchis* to make diel vertical migrations (Chapter, 2).

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VI. BUOYANCY EXPERIMENTS

Differential diel sinking rates may facilitate diel vertical migration of *Polyorchis penicillatus*

Introduction

Many gelatinous zooplankters move about the water column by active swimming. However, some planktonic hydrozoans use other devices such as lipid accumulation, gas floats, or ion exclusion (Mackie 1974) to provide lift which augments swimming. The latter mechanism, ion exclusion, is well known in hydromedusae and many of them can alter their buoyancy and can regain their "normal" buoyancy state within a few hours after encountering hypo- or hypertonic seawater (Leonard 1980; Mills 1984). This density regulating ability is probably an adaptation to the estuarine conditions in which many hydromedusae are found. However, changes in buoyancy may also be important during periods when large water column distances are to be travelled, such as during diel vertical migration and when changes in density occur due to feeding. These changes may compound the effects of swimming alone (Eyden 1923; Mackie 1974). Mills and Vogt (1984) looked at a variety of hydromedusae which make diel migrations and their diel patterns of ionic composition as an indication of density changes. They found that mesogleal ions, which have most often been implicated in controlling buoyancy, did not show any diel differences in concentration. Thus, they concluded that ionic changes, and by extrapolation buoyancy changes, did not contribute to diel migration and that their migrations were due to changes in swimming activity alone.

Mechanisms other than ionic exchange may, however, alter buoyancy and a more direct, simplistic approach to this problem, would be to look at diel density changes of individuals. Precise measurements of changes in the absolute density of individuals is very difficult, but because the sinking rate of an individual is a fairly good indication of its density with respect to the surrounding medium, any diel differences in sinking rate should suggest some changes in buoyancy compensation, regardless of the mechanism. The purpose of this study was to compare the sinking rates of various sizes of daytime and nighttime adapted *Polyorchis*. Results from this study show a significantly greater sinking rate for daytime adapted individuals than for nighttime adapted ones. These data suggest that the diel vertical migration of *Polyorchis*, which is initiated by the shadow response, may be facilitated by corresponding buoyancy changes.

Methods and Materials

Fifty-five *Polyorchis* of various sizes (1.1 - 3.8 cm BH) were collected from Bamfield Inlet, Bamfield, B.C. during June 1983. Medusae were kept unfed in running sea water under natural photoperiod for several days before beginning experiments. Immediately before each experiment, four radial cuts were made through the bell margin and up the swimming muscle; this prevented effective swimming. Each individual was then placed just below the surface of the water and released. The sinking rate was determined as the total time each individual required to sink 20 cm. The mean sinking rate for each individual medusa was determined after 10 separate trials. Sinking rates for 25 medusae tested from 13:00-16:00 (daytime) and 30 medusae tested from 01:00-04:00 (nighttime) on several separate dates were determined.

Medusae were maintained under the same sea water conditions as those used to measure sinking rates. No attempt was made to standardize the temperature / salinity conditions either from day to day trials or from day to night trials. However, sea water conditions varied little between day and night due to the depth of the sea water intakes in Bamfield (e.g., 27 June 01:00, 11.2 °C, 30.0‰; 27 June, 14:00, 13.2 °C, 29.1‰).

Results

Medusae sank at rates ranging from 18 cm/min to greater than 60 cm/min with smaller individuals sinking slightly slower than larger individuals (Fig. 37), however regression analysis (Sokal and Rohlf 1969) showed that this relationship was not significant ($p > 0.05$) for either day- or nighttime adapted individuals. Furthermore, there was a significant ($p < 0.01$) difference between the 23 mean daytime sinking rates and between the 23 means for the nighttime. Based on these findings, I pooled all daytime and nighttime individuals into 2 groups. Pooled daytime individuals showed a mean (± 1 SE) sinking rate of 42.3 (0.66) cm / min ($n=230$), while nighttime individuals sank at 39.2 (0.66) cm / min ($n=230$). A one-way ANOVA comparing pooled day- and nighttime sinking rates showed that these mean sinking rates are significantly ($0.01>p>0.001$) different. This was based on $a(n-1)$ df where $a=2$ (day and night) and $n=23$. In addition, 7 / 30 (23.3%) nighttime individuals were either neutrally or positively buoyant and did not sink within five minutes after the start of the experiment. Only 2 / 25 (8.0%) daytime individuals were either neutral or positively buoyant.

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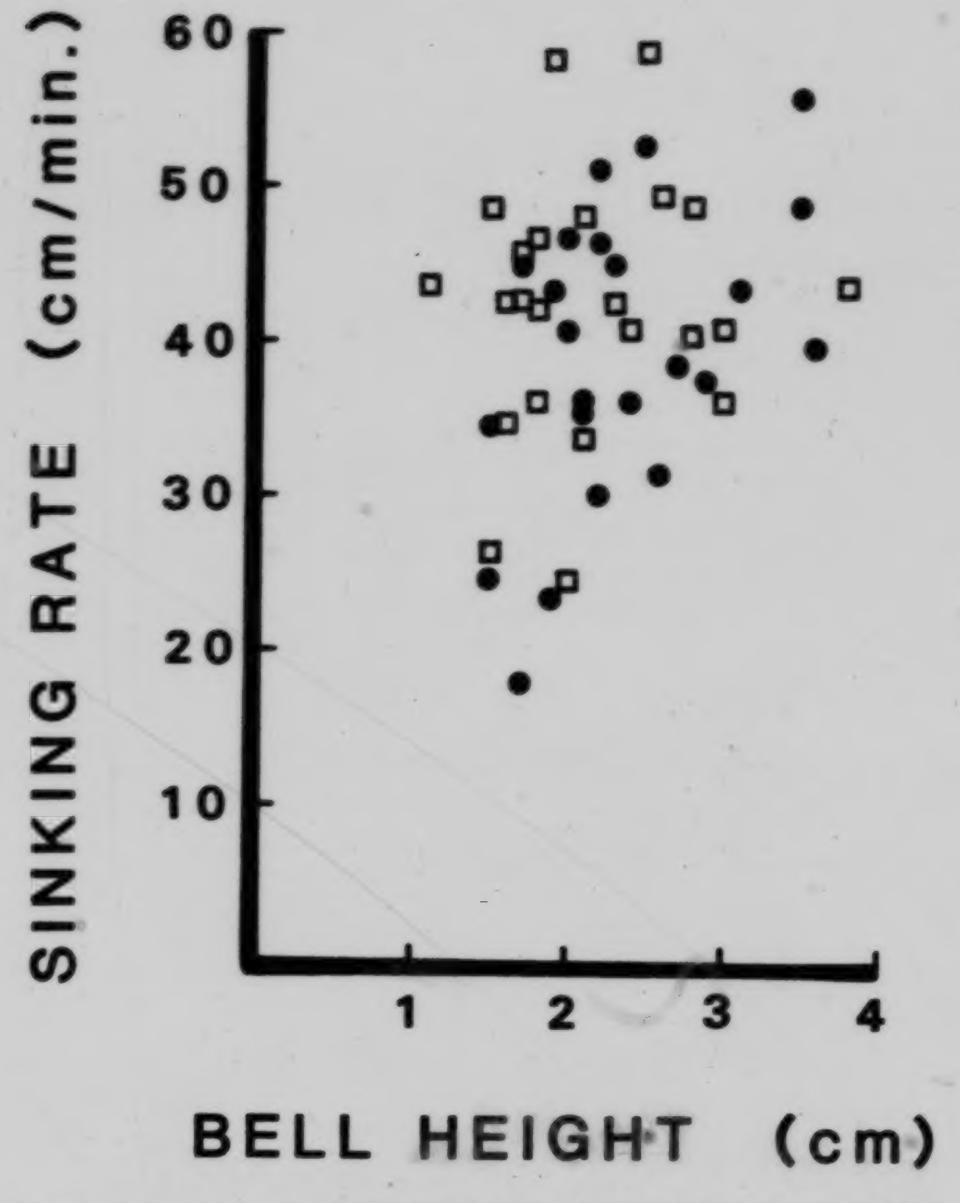

Figure 37. Plot of the mean sinking rates of various sizes of *Polyorchis* during daytime (□) and nighttime (●).

Discussion

The results from this study have shown that individuals of *Polyorchis* under daytime conditions sink at a greater rate than those under nighttime conditions. This day/night difference in sinking rate suggests that *Polyorchis* can control its density with respect to sea water and become more negatively buoyant during the daytime and less negatively buoyant at night. Although it has been shown that hydromedusae can alter their buoyancy when placed in hyper- or hypotonic sea water (Leonard 1980; Mills 1984), the mechanism of these buoyancy changes has not been conslusively demonstrated. Mills and Vogt (1984) have shown that the mesogleal concentration of ions suspected to be most important in buoyancy compensation (i.e., SO_4^{2-}, Na^+, Mg^{2+}, K^+, Ca^{2+}) (Denton and Shaw 1961; Mackay 1969; Bidigare and Biggs 1980) did not show any significant diel differences in mesogleal ionic concentrations in several species of diel migrating hydromedusae, including *Polyorchis*. However, Mills and Vogt's (1984) study included *Polyorchis* in all of the ion analyses with the conspicuous exception of sulphate. In her thesis, Mills (1982) did look at sulphate concentration for *Polyorchis* and found a significantly greater concentration of sulphate in daytime animals than nighttime animals, but she stated (pers. comm.) that she did not include these data in the publication (Mills and Vogt 1984) because there was a great deal of variability and was doubtful of their accurracy. In addition, a gravimetric analysis of mesogleal sulphate concentration in collaboration with Dr. W.C. Mackay (Univ. of Alberta) showed that 8 daytime adapted individuals of *Polyorchis* had a mean (±1 SE) sulphate concentration of 5.140 (0.561) mM / l of mesoglea. Eight nighttime adapted individuals had 6.085 (0.807) mM / l of mesoglea. A one-way ANOVA (Sokal and Rohlf 1969) showed that these means were not significantly ($p > 0.05$) different. These values were considerably lower than the concentration of sulphate ion in the seawater at night (25.20 mM / l) and day (25.84 mM / l). Conflicting results from this study and Mills' unpublished thesis work preclude any decision about whether sulphate is responsible for bouyancy changes in *Polyorchis*. The mechanisms of buoyancy changes in *Polyorchis* is beyond the scope of this thesis and remains equivocal, but the sinking rate data from this study do suggest that *Polyorchis* does undergo slight diel changes in its buoyancy which is seen as differential sinking rates.

The differential sinking rate of *Polyorchis* may facilitate its diel migration by augmenting the primary mode of locomotion, swimming. The greater positive lift at night should exaggerate the results of upward swimming caused by the shadow response. The combined effects of increased swimming frequency and slower sinking should result in a sustained higher position in the water column. At dawn, the combined effects of increased density, crumpling, and reduced swimming should result in a net downward movement and an accumulation of individuals near the bottom. This is what is observed in the field (Chapter 2). It is presently unknown what triggers buoyancy changes, but the rapidly changing light conditions at dusk and dawn, which cause the shadow response, may also be important in triggering buoyancy changes.

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VII. CONCLUSION

The purpose of this thesis was to characterize the photic behavior of the hydromedusan *Polyorchis penicillatus* and to relate this behavior to its diel patterns of activity in the field. I have done this by using a variety of techniques and this multi-discipline approach to various questions has enabled me to corroborate findings at various levels and to ask a broader range of questions. In this final chapter, I will briefly review some of the major findings in each chapter and relate these findings to each other.

In Chapter 2, I described the diel vertical migration and other behavior of *Polyorchis* in the field. It is clear that *Polyorchis* moves up into the water column at sunset and moves down toward the bottom at dawn. My behavioral observations of swimming activity showed that *Polyorchis* swims at very low frequencies most of the time; this I dubbed "maintenance swimming". The significance of the diel vertical migration by *Polyorchis* is suggested by the concommitant diel vertical migration of many demersal plankter taxa; the principle prey of *Polyorchis*. Documenting that *Polyorchis* did indeed make a distinct diel vertical migration was an important initial step in the thesis. From this point onward, I designed various experiments to explain the mechanisms of this coordinated movement.

The whole animal experiments in Chapter 3 were designed to test the various photic response of *Polyorchis* and to detemine if any of these could explain the diel vertical migration. I tried to simulate the photic stimuli that *Polyorchis* might encounter in the field and therefore, in these and all experiments throughout the thesis, photic stimuli were biologically meaningful with intensities representative of those found under field conditions. In this chapter, I showed that slow, continuous changes in light intensity were most important in altering "maintenance swimming" frequency. These changes in swimming frequency probably occur during sunset and sunrise and may initiate the upward and downward movements of *Polyorchis* in the water column. It is important to note here that although the changes in "maintenance swimming" frequency probably occurs during periods of the most rapid change in light intensity (e.g., sunset and sunrise), the effect is transient and can not explain the maintained nighttime and daytime positions. However, in Chapter 6, I have provided evidence that suggests that *Polyorchis* undergoes diel buoyancy changes. These changes may be triggered by the rapid changes in light intensity and appear to be long-lived. In addition to augmenting the initial movements at sunset and sunrise, these buoyancy changes may explain the maintained daytime and nighttime positions. The mechanisms of these changes is not known, but further work in this area may be fruitful.

I have also shown that the responses to rapid reductions in light intensity (the shadow response) were short-lived and did not result in marked changes in swimming frequencies and therefore could not result in large movements. Thus, the shadow response, which has been traditionally been thought to be used in predator avoidance, could not perform this function. I have also demonstrated that a shadow of the magnitude and duration that might be generated by predators could not produce effective escape swimming (Chapter 5).

In Chapters 4 and 5, I attempted to describe the photic behavior of *Polyorchis* (as demonstrated in Chapters 2 and 3) in terms of the cellular properties of the various components involved in the shadow response. Here I demonstrated that the shadow response is a simple reflex in the strictest sense of the word. The three criteria I used to describe a reflex were: 1) a predictable sequence of events. This is summarized in Figure 22 and shows that the first event in response to a shadow is the hyperpolarization of the "O" system. This is followed by spiking in the SMNs and "B" system which produces swimming and tentacle myoepithelium contractions, respectively.; 2) morphologically and physiologically identifiable reflex arc components. These are summarized in Figures 11 and 12. I have presented evidence here that the "O" system may be the primary photoreceptor and afferent neurons and function as a centralized integrator of photic information. The efferent neurons, SMNs and "B" system, drive the effector organs, swimming and tentacle myoepithelium, respectively.; 3) a graded response with respect to the intensity of a specific stimulus. I have shown that the "O" system shows a graded hyperpolarization that is proportional to the rate of decrease in light intensity and that the SMN firing rate is also graded with this rate of change.

Although reference to the shadow response as a reflex may suggest to some readers a reversion to the "chain reflexes" idea, using the word reflex to describe a simple behavior in a hydromedusan may advance our thinking about cnidarian nervous systems and behavior. There are at least two reasons why I think this is so. First, the word reflex connotes very specific ideas about the nervous organization and neuronal mechanisms which heretofore has not really been considered for hydromedusae. This is probably due to the late intervention of intracellular techniques and thus a general ignorance of cnidarian neuronal mechanisms. However, marked changes in the understanding of cnidarian neurobiology have resulted and will continue with the relatively recent advent of intracellular techniques on both scyphomedusae and hydromedusae. The use of these techniques and the results from such studies has begun to force cnidarian neurobiologists to divorce themselves from extracellular recordings. The terminology of intracellular techniques is more precise and more importantly, is universal among neurobiologists. Extracellular recordings from various cnidarians, which referred to some activity as a "marginal pulse" or "tentacle pulse", are very confusing and I think this, together with the lack of identifiability of neuronal components, created a credibility problem for cnidarian neurobiology. However, with the current use of intracellular techniques, cnidarian neurobiologists can now talk in terms of basic cellular properties which appear to be conserved throughout all neuronal systems. Now that cnidarian neurobiologists are beginning to speak a more universal language, there is an irresistable urge to make comparisons between the neuronal mechanisms of cnidarian systems and more complex systems. My attempt to make an anology between a simple form of integration of photic information in the vertebrate retina and the electrically-coupled "O" system and ocelli is one such comparison. This seemingly backward comparison from simple to more complex neuronal systems may be the rule for some time until more intracellular information from cnidarians is available. However, with the ever increasing intracellular knowledge of the mechanisms of cnidarian nervous systems, those working in more complex systems may begin reducing or simplifying their systems and using cnidarian neuronal properties as examples.

The second reason that using the word reflex to descibe a behavior will change conventional wisdom is that reference to a simple behavior as a reflex suggests that a hierarchy of behavior does exist in simple animals and that more complex behavior is possible. That more complex behavior is possible is suggested through the field and laboratory observations of *Polyorchis*. One of these is the apparent spontaneous extended swimming bouts described in Chapter 2. This behavior may result from some kind of "decision" to move from areas of low prey encounters. Other behavior, most notably those involved in feeding, may include complex coordination of tentacles, manubrium, and bell margin.

It is clear from this study that one can fully characterize a simple behavior, such as a reflex, in a simply organized animal. Whether one will be able to describe more complex behavior remains to be seen, however, medusae will be good candidates for such work.